Inventor: Schwiebert et al.
Title: Methods and Compositions for P2X Receptor Calcium Entry Channels and Other Calcium Entry Mechanisms
Docket No.: 21085.0044U3
REPLACEMENT SHEET

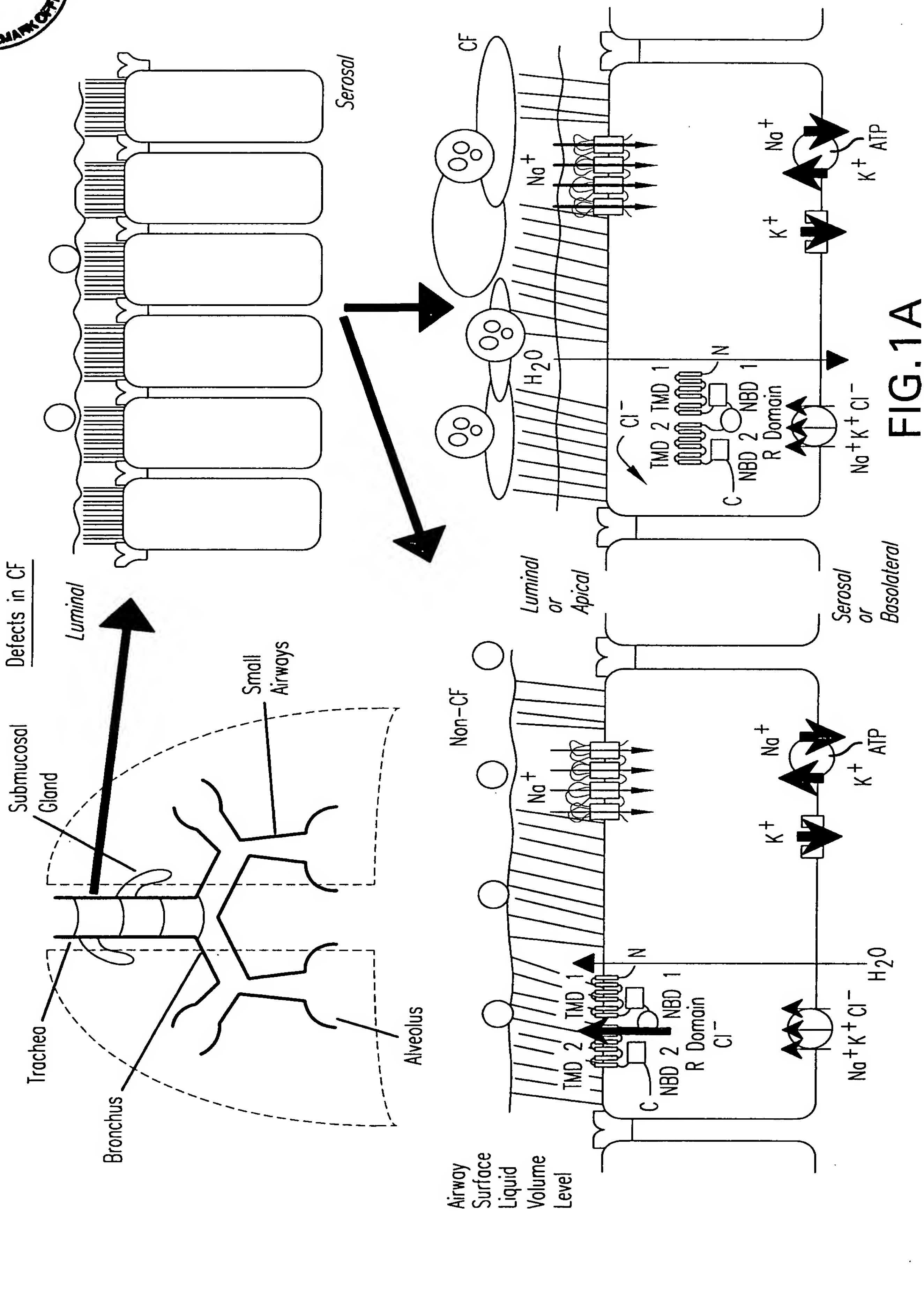


FIG.1A

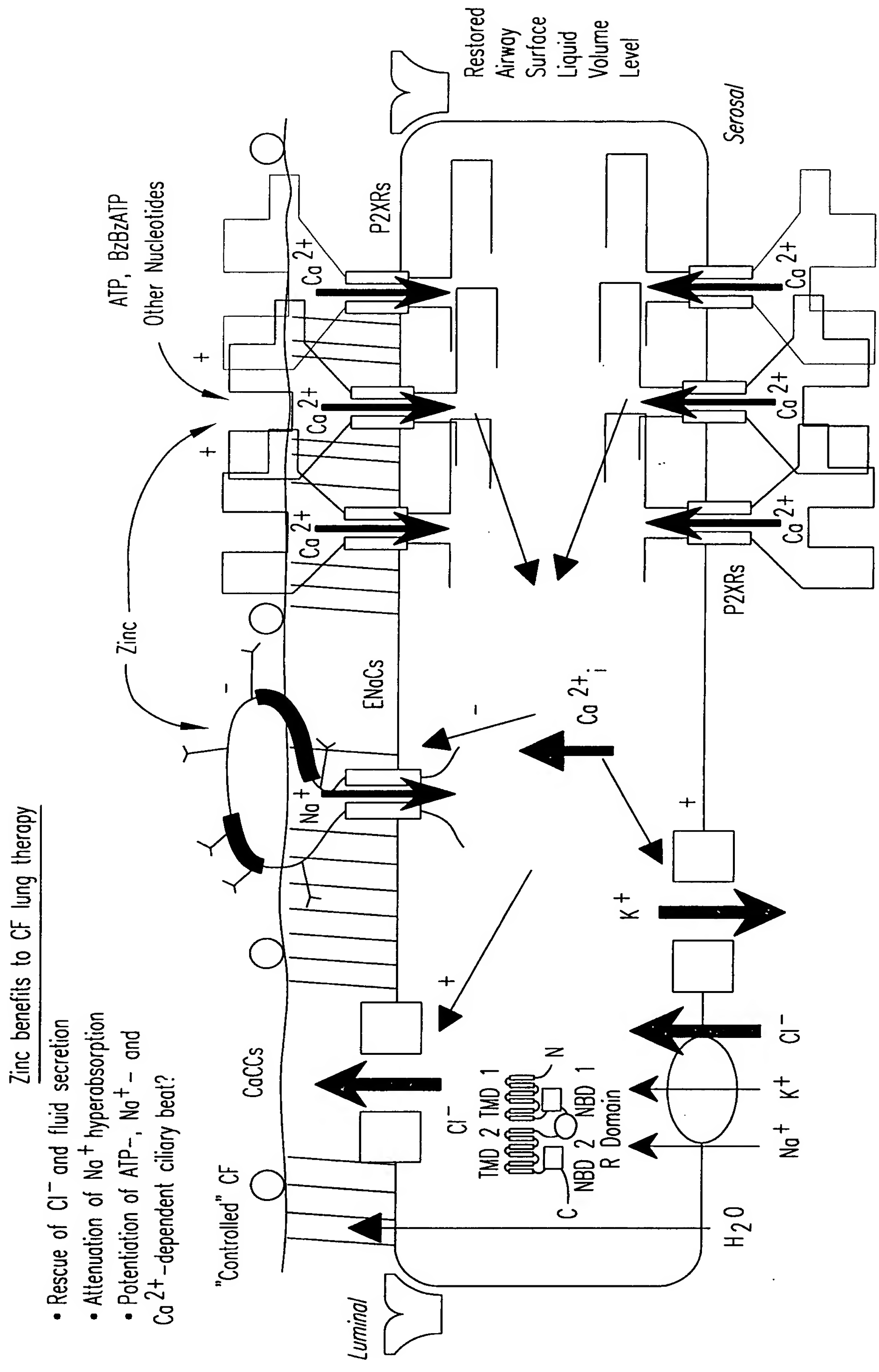

Zinc benefits to CF lung therapy
• Rescue of Cl− and fluid secretion
• Attenuation of Na+ hyperabsorption
• Potentiation of ATP–, Na+– and Ca 2+–dependent ciliary beat?
"Controlled" CF
Luminal
CaCCs
Cl−
TMD 2 TMD 1
N
C
NBD 2 NBD 1
R Domain
H2O
Na+
K+
Cl−
K+
Zinc
ENaCs
Na+
Ca 2+ i
ATP, BzBzATP
Other Nucleotides
Ca 2+
P2XRs
Restored
Airway
Surface
Liquid
Volume
Level
Serosal

FIG.1B

Zinc as an anti-inflammatory for CF and other airway diseases such as asthma and common cold

- Zinc in a solution-based formulation enters the cell as free ionic zinc and inhibits Zinc NFkappaB activation

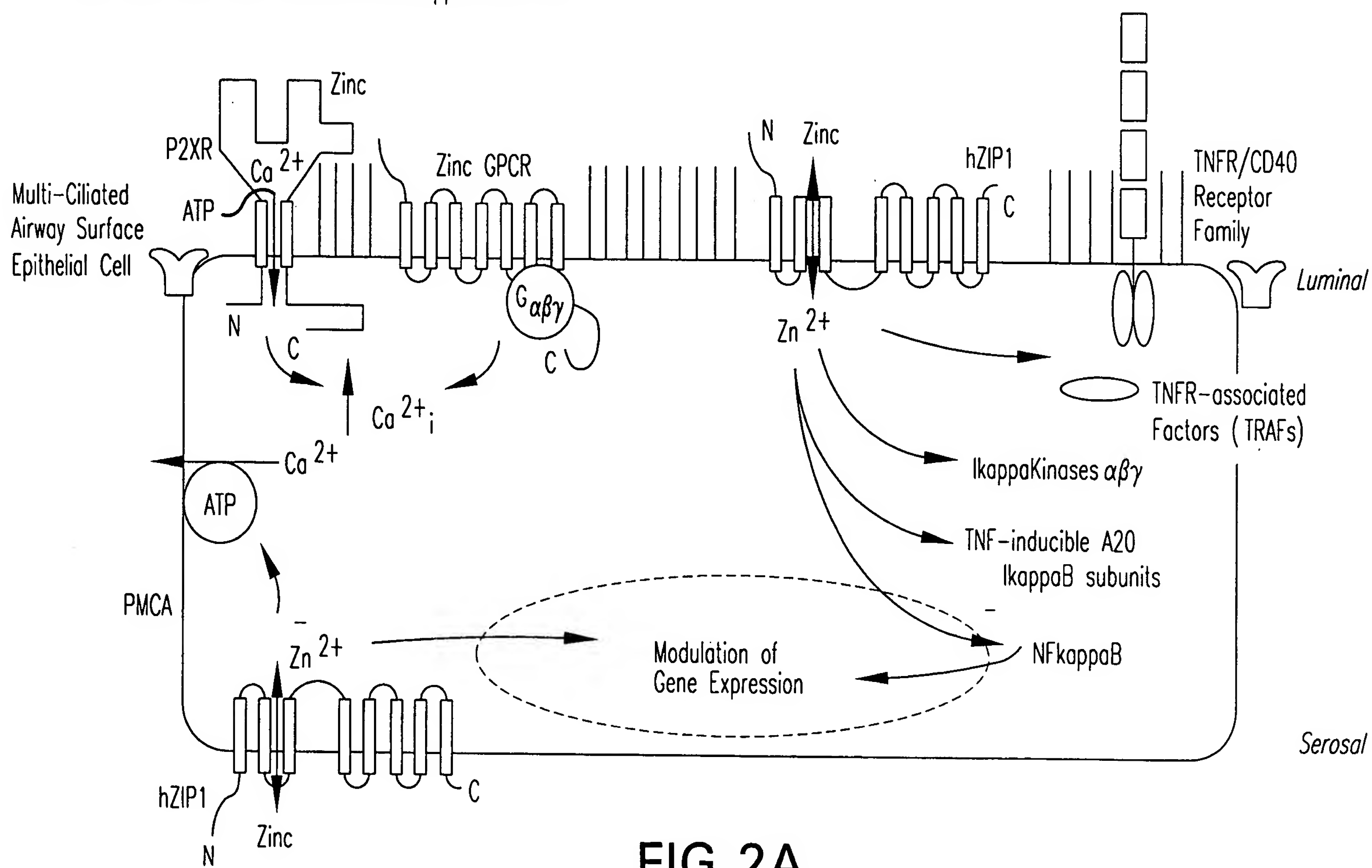


FIG.2A

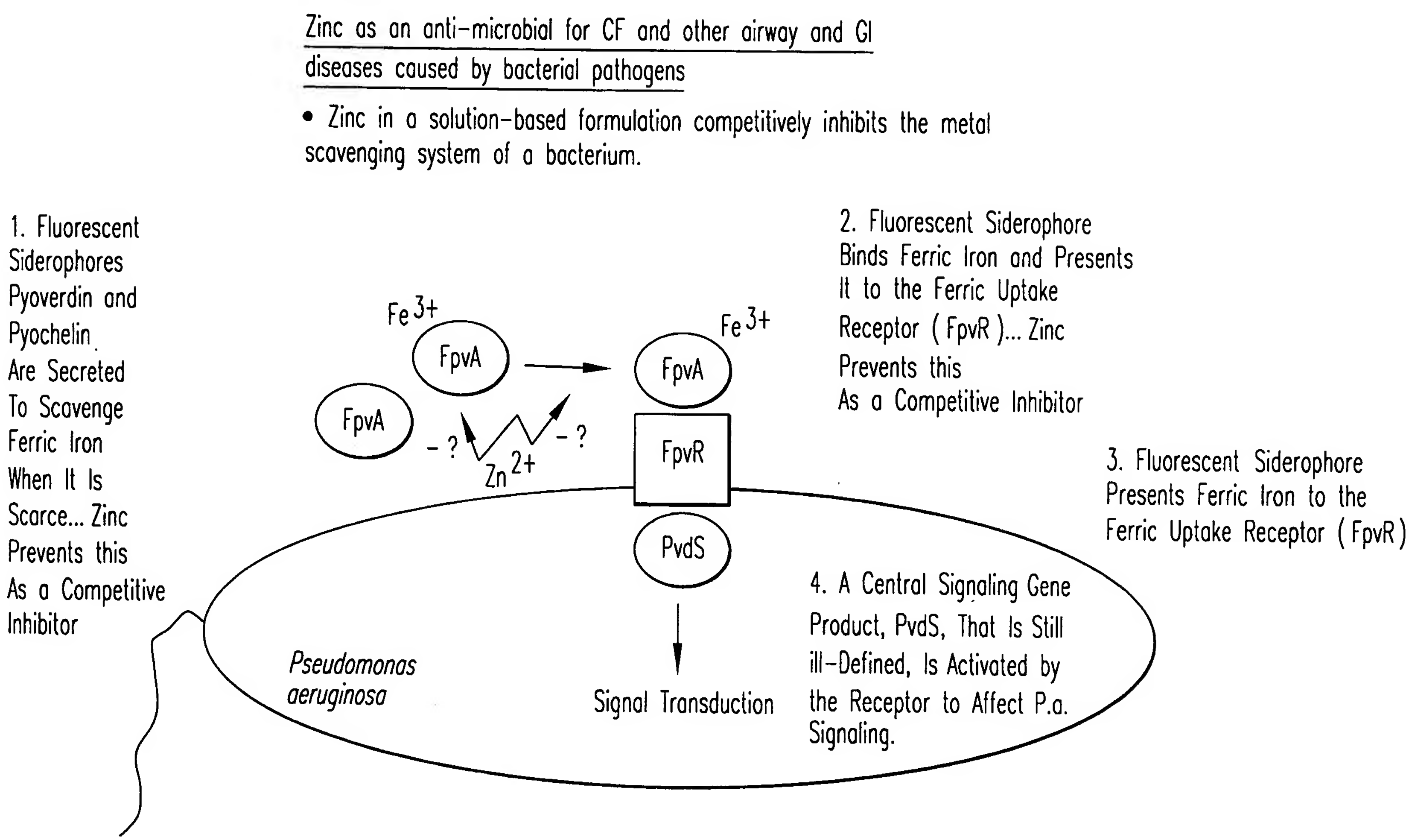

Zinc as an anti-microbial for CF and other airway and GI diseases caused by bacterial pathogens
• Zinc in a solution-based formulation competitively inhibits the metal scavenging system of a bacterium.
1. Fluorescent Siderophores Pyoverdin and Pyochelin Are Secreted To Scavenge Ferric Iron When It Is Scarce... Zinc Prevents this As a Competitive Inhibitor
Fe 3+
FpvA
FpvA
- ?
Zn 2+
- ?
Fe 3+
FpvA
FpvR
PvdS
Signal Transduction
Pseudomonas aeruginosa
2. Fluorescent Siderophore Binds Ferric Iron and Presents It to the Ferric Uptake Receptor (FpvR)... Zinc Prevents this As a Competitive Inhibitor
3. Fluorescent Siderophore Presents Ferric Iron to the Ferric Uptake Receptor (FpvR)
4. A Central Signaling Gene Product, PvdS, That Is Still ill-Defined, Is Activated by the Receptor to Affect P.a. Signaling.

FIG.2B

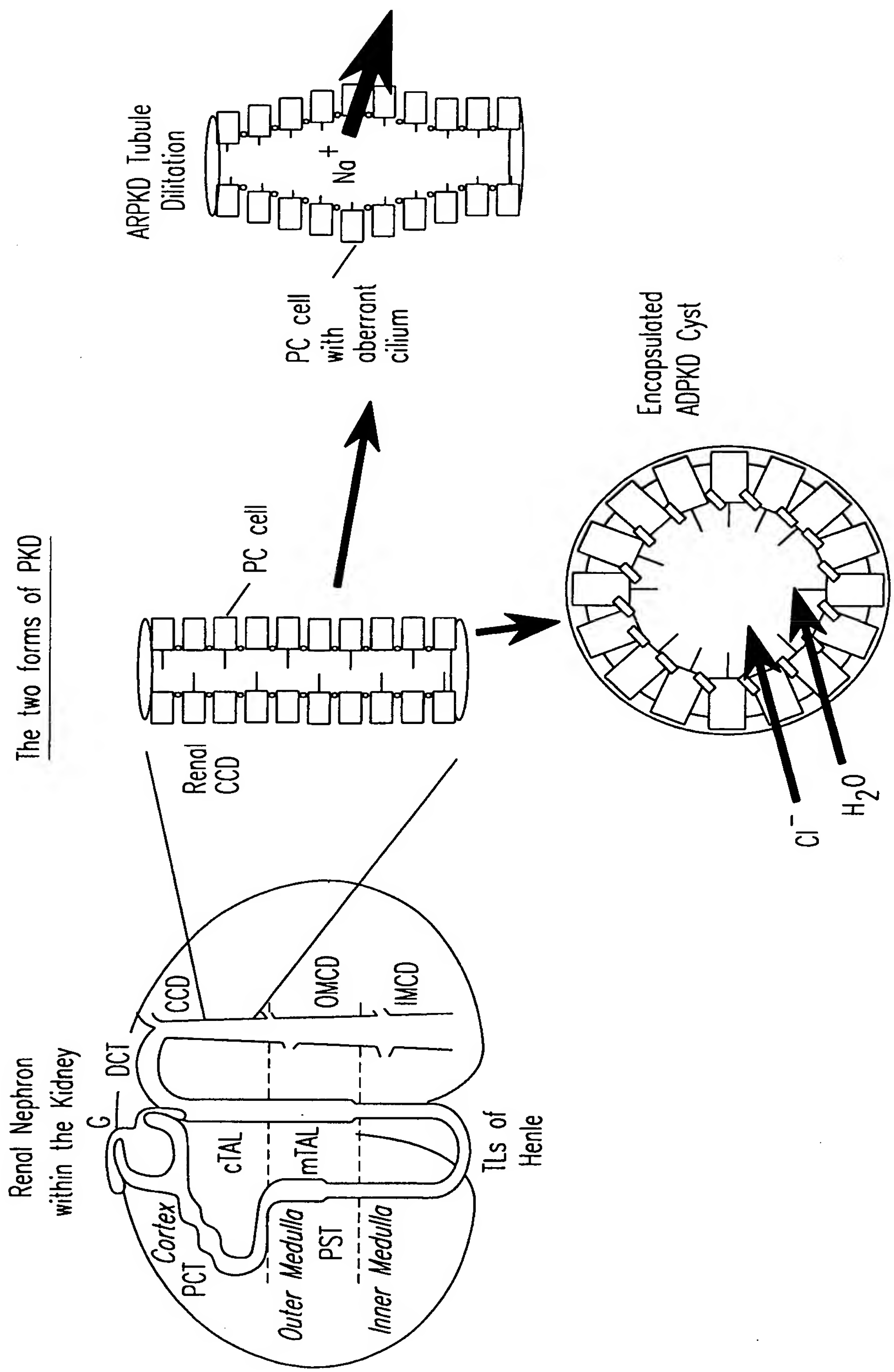

The two forms of PKD
Renal Nephron within the Kidney
G
DCT
CCD
OMCD
IMCD
Cortex
PCT
cTAL
mTAL
Outer Medulla
PST
Inner Medulla
TLs of Henle
Renal CCD
PC cell
ARPKD Tubule Dilitation
Na^+
PC cell with aberrant cilium
Encapsulated ADPKD Cyst
Cl^-
H_2O

FIG.3A

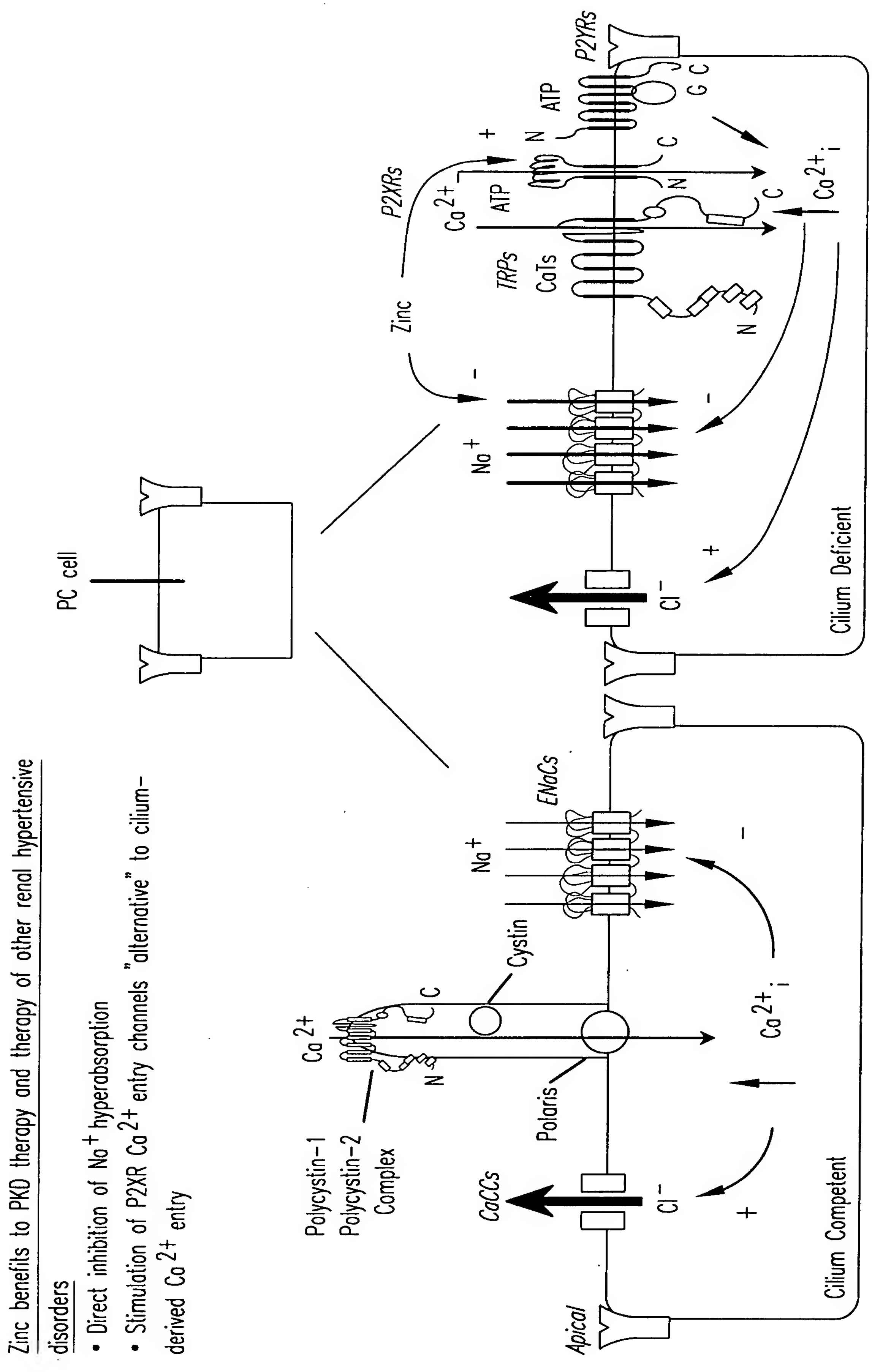

Zinc benefits to PKD therapy and therapy of other renal hypertensive disorders
• Direct inhibition of Na+ hyperabsorption
• Stimulation of P2XR Ca 2+ entry channels "alternative" to cilium-derived Ca 2+ entry
PC cell
Apical
Polycystin-1
Polycystin-2
Complex
Ca 2+
N
C
Polaris
Cystin
CaCCs
Cl −
+
Ca 2+ i
Na+
ENaCs
−
Cilium Competent
Zinc
P2XRs
Ca 2+
ATP
TRPs
CaTs
N
C
+
N
ATP
P2YRs
G C
Ca 2+ i
−
Na+
+
Cl −
Cilium Deficient

FIG.3B

Normal Insulin Secretion in a Pancreatic Islet β Cell

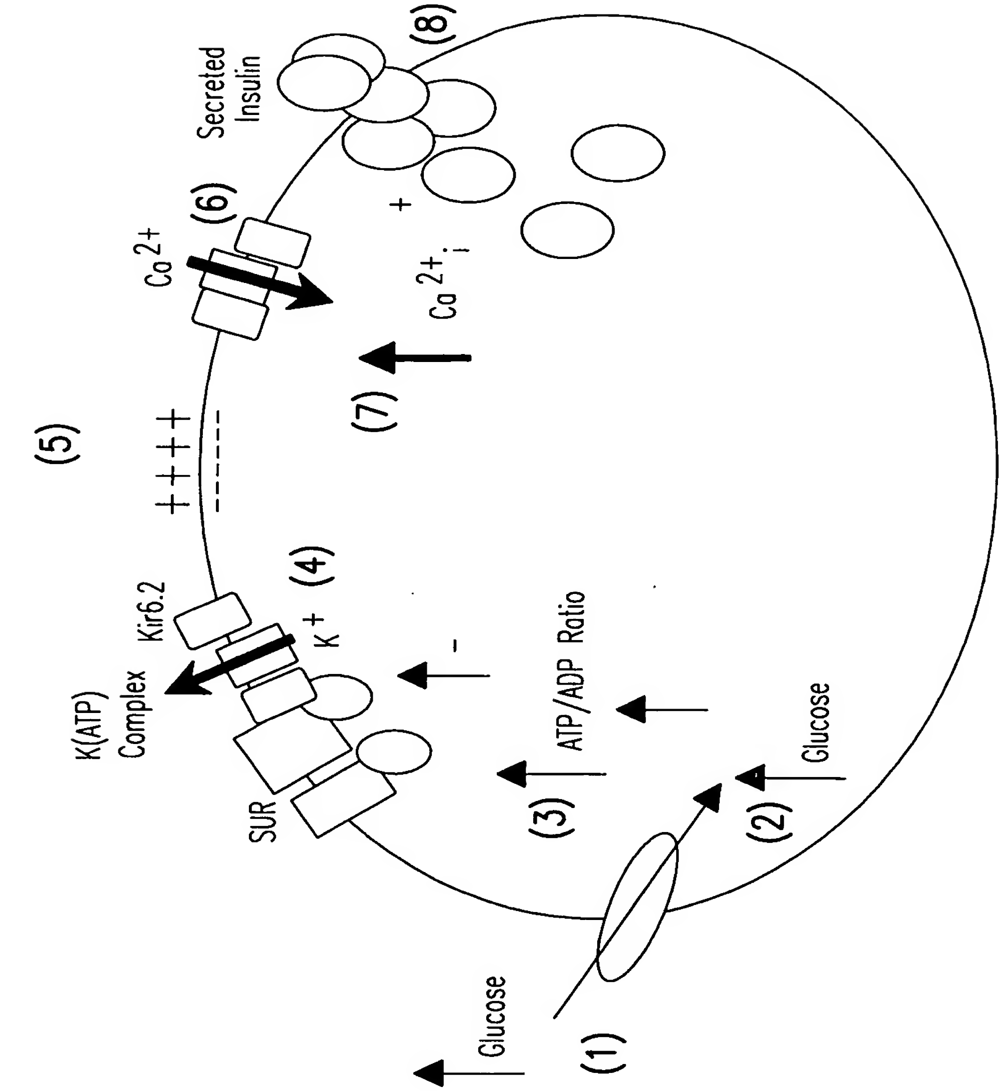


FIG.4A

"Controlled" Diabetic β Cell

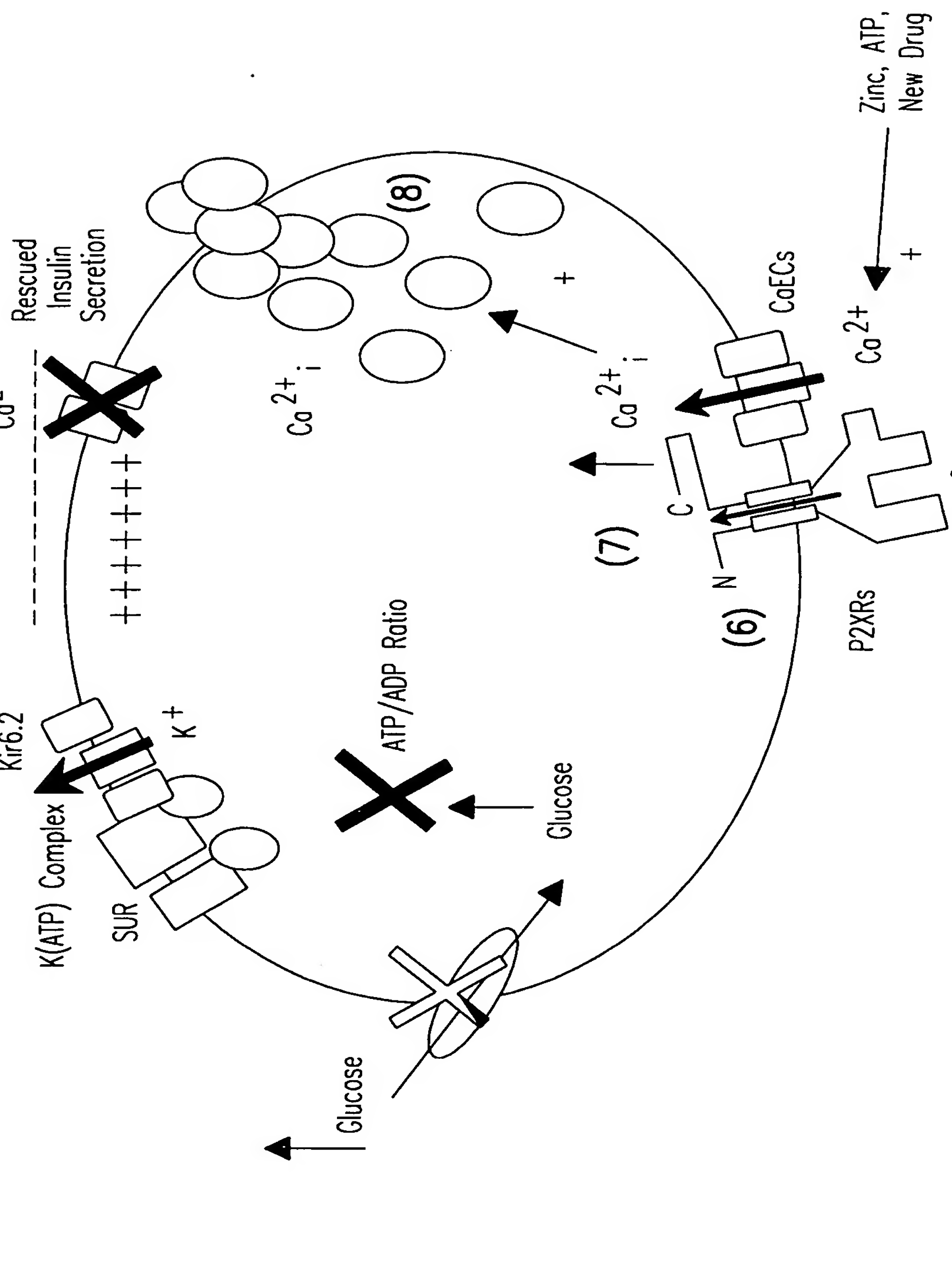


FIG.4B

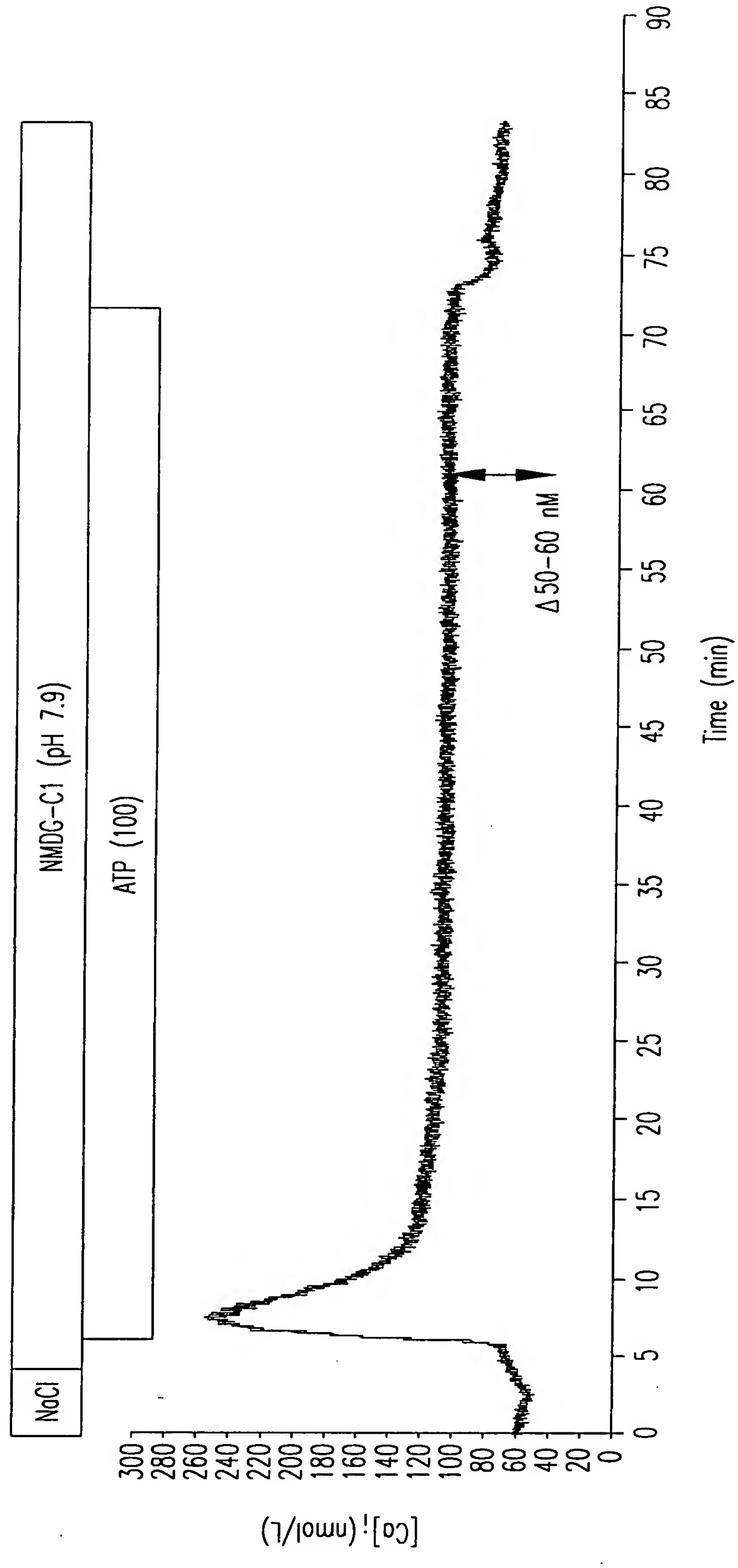

NaCl
NMDG-C1 (pH 7.9)
ATP (100)
Δ50–60 nM
[Ca]i (nmol/L)
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
Time (min)

FIG.5A-1

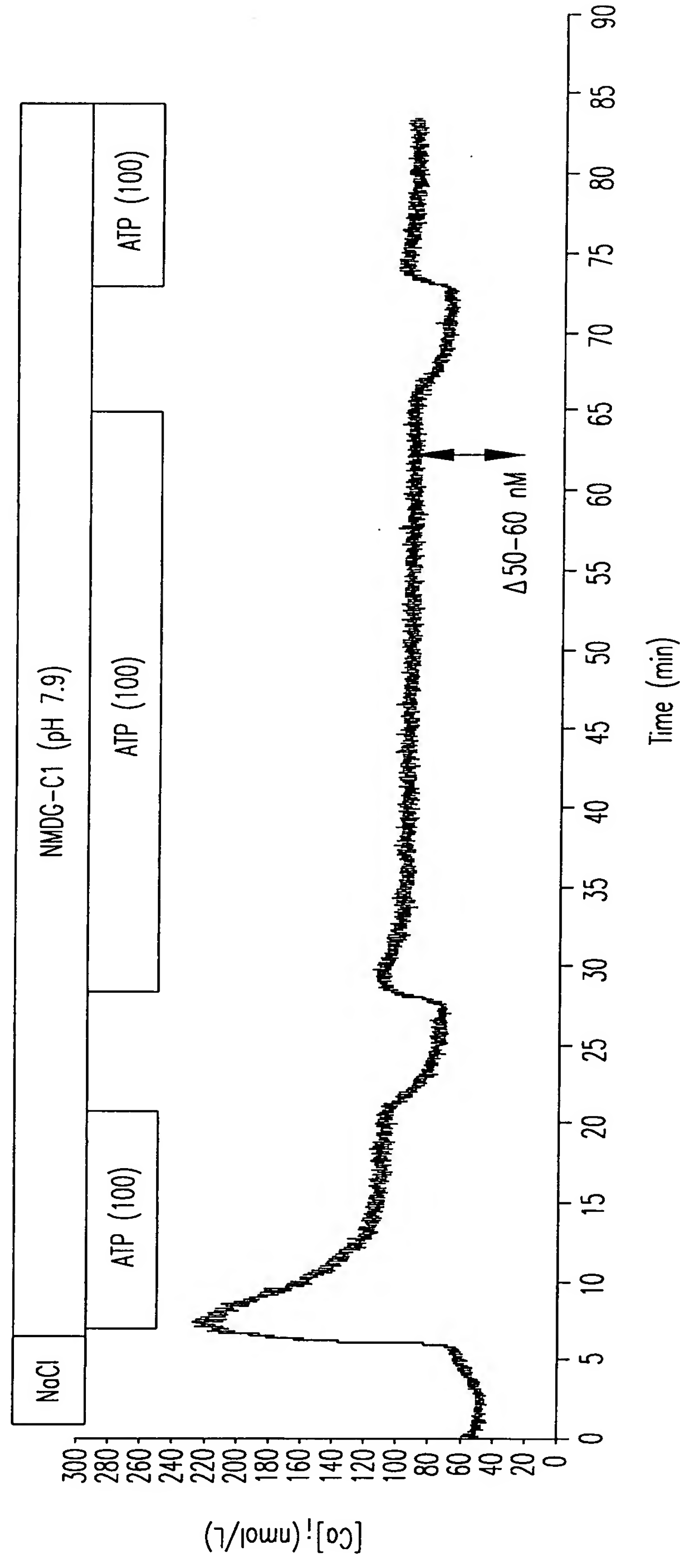

NaCl
NMDG-C1 (pH 7.9)
ATP (100)
ATP (100)
ATP (100)
Δ50-60 nM
[Ca]$_i$ (nmol/L)
Time (min)
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300

FIG.5A-2

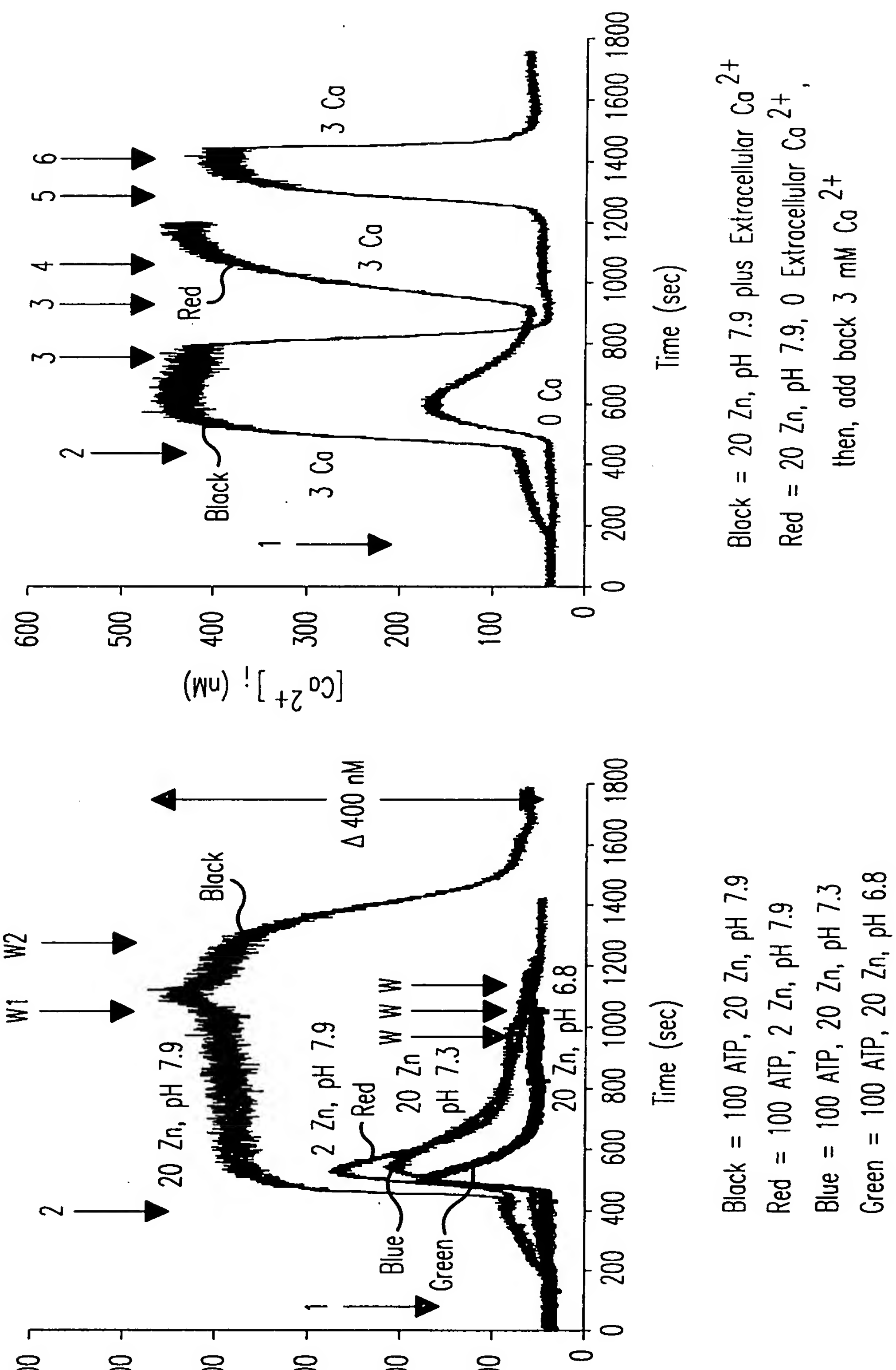

[Ca 2+]i (nM)
Time (sec)
W1
W2
2
1
20 Zn, pH 7.9
Black
Δ 400 nM
2 Zn, pH 7.9
Red
Blue
Green
20 Zn pH 7.3
W W W
20 Zn, pH 6.8
Black = 100 ATP, 20 Zn, pH 7.9
Red = 100 ATP, 2 Zn, pH 7.9
Blue = 100 ATP, 20 Zn, pH 7.3
Green = 100 ATP, 20 Zn, pH 6.8
3
3
4
5
6
Red
3 Ca
0 Ca
Black = 20 Zn, pH 7.9 plus Extracellular Ca 2+
Red = 20 Zn, pH 7.9, 0 Extracellular Ca 2+ ,
then, add back 3 mM Ca 2+

FIG.5B

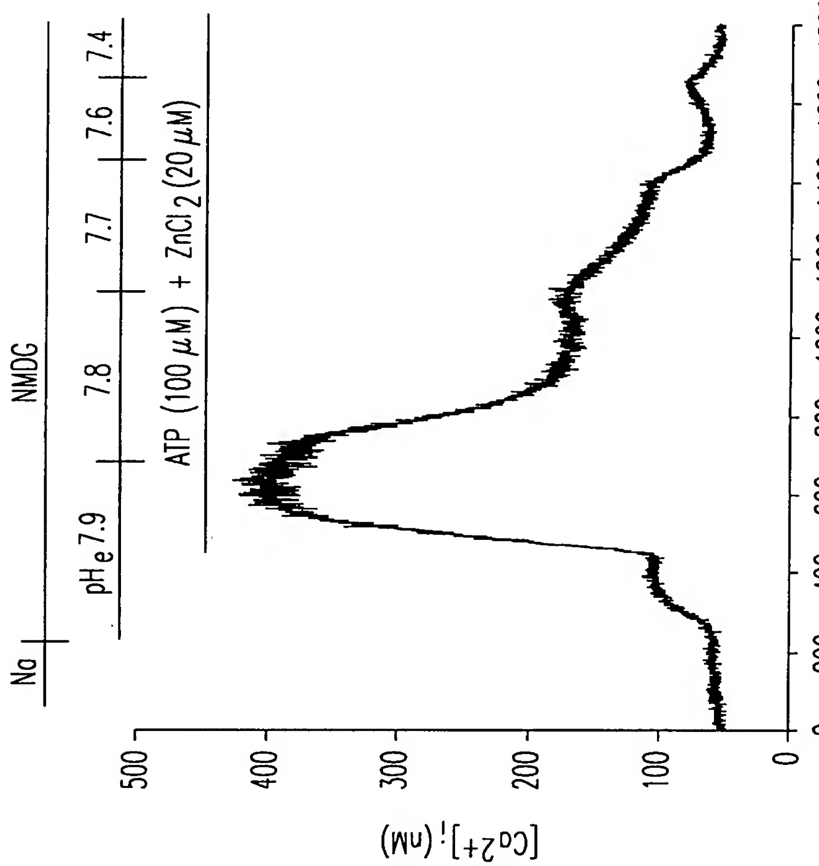

Na
NMDG
pHe 7.9
7.8
7.7
7.6
7.4
ATP (100 μM) + ZnCl2 (20 μM)
[Ca2+]i (nM)
500
400
300
200
100
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time (s)

FIG.5D

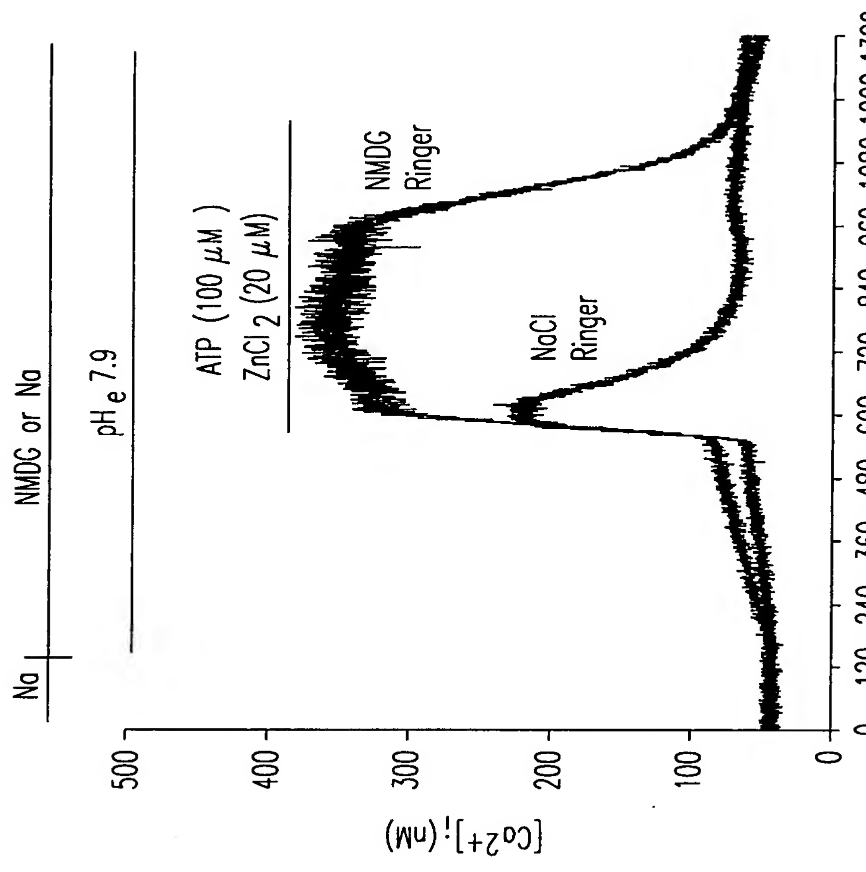

Na
NMDG or Na
pHe 7.9
ATP (100 μM)
ZnCl2 (20 μM)
NMDG
Ringer
NaCl
Ringer
[Ca2+]i (nM)
500
400
300
200
100
0
0
120
240
360
480
600
720
840
960
1080
1200
1320
Time (s)

FIG.5C

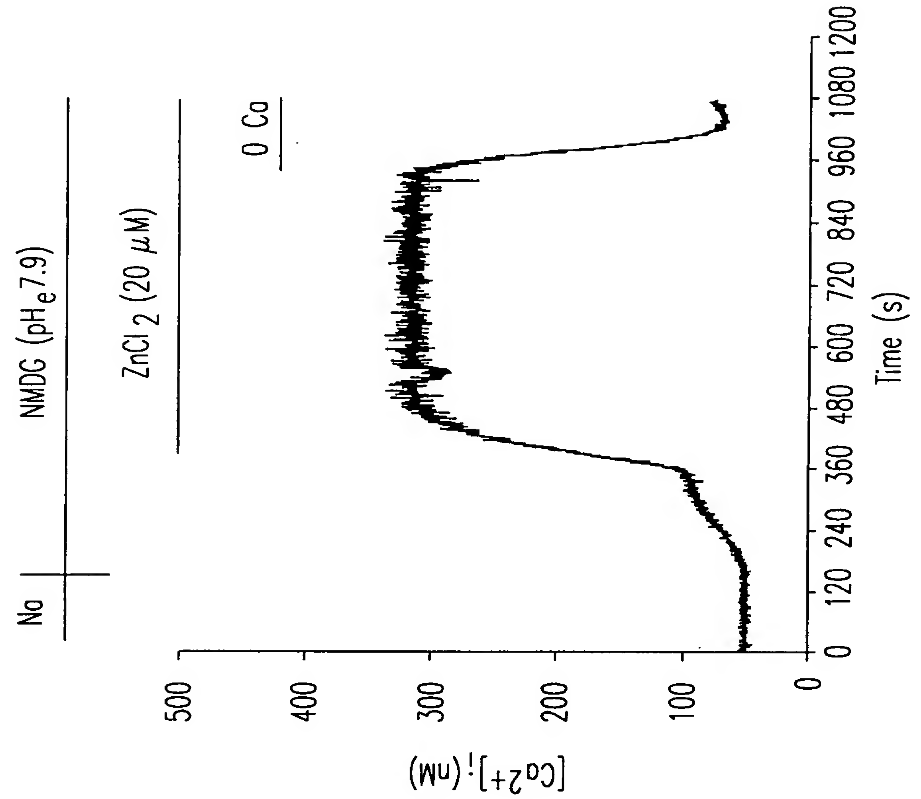

Na
NMDG (pH$_e$ 7.9)
ZnCl$_2$ (20 μM)
0 Ca
500
400
300
200
100
0
[Ca^{2+}]$_i$ (nM)
0 120 240 360 480 600 720 840 960 1080 1200
Time (s)

FIG.5F

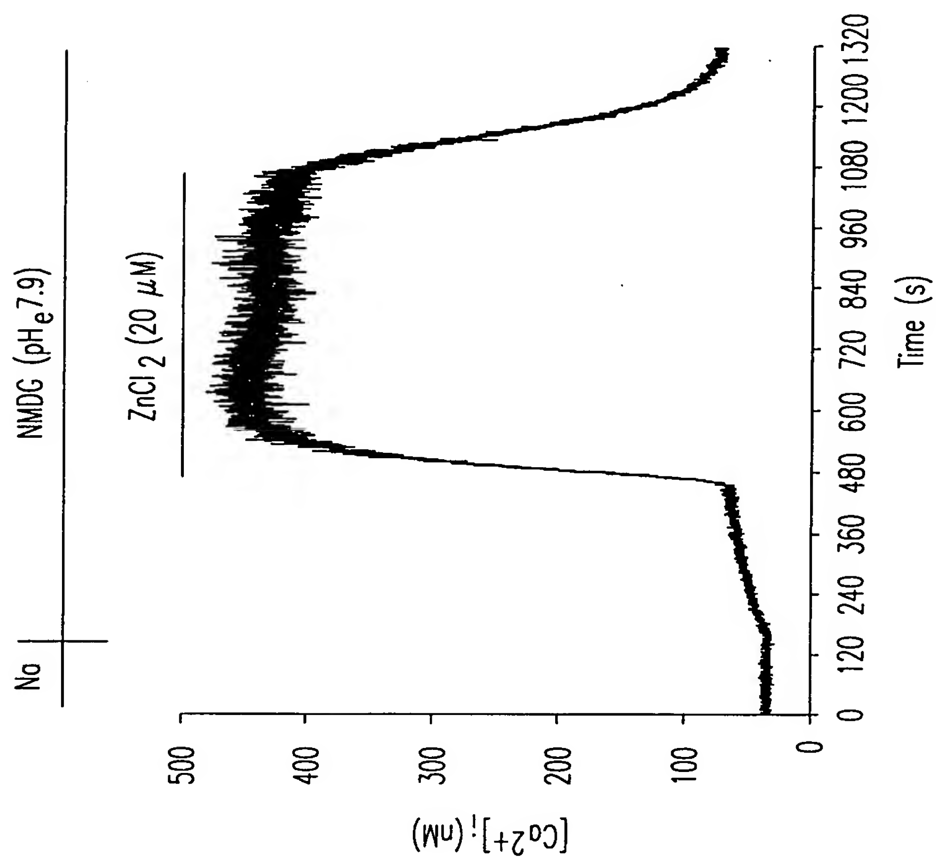

Na
NMDG (pH$_e$ 7.9)
ZnCl$_2$ (20 μM)
500
400
300
200
100
0
[Ca^{2+}]$_i$ (nM)
0 120 240 360 480 600 720 840 960 1080 1200 1320
Time (s)

FIG.5E

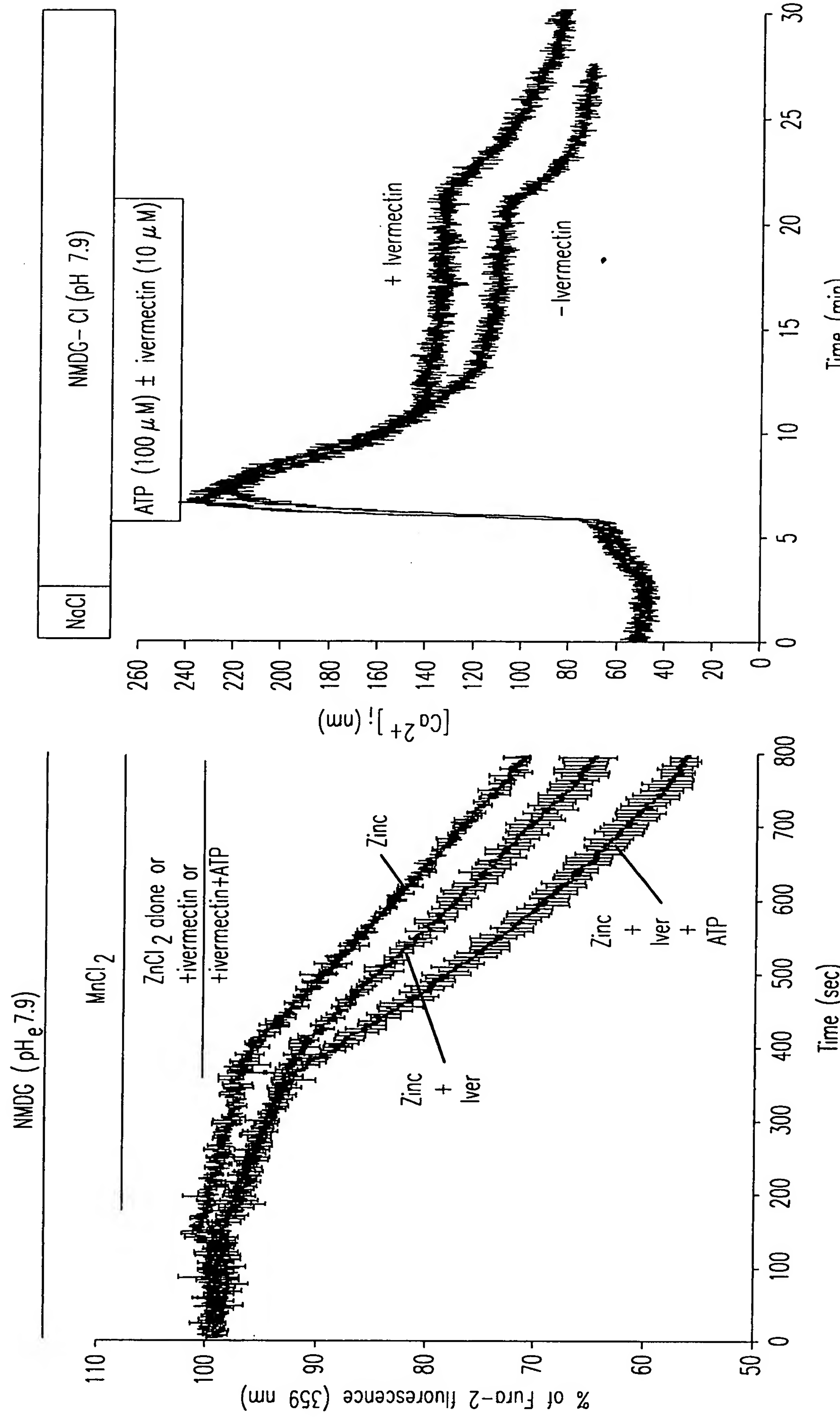

NMDG (pH_e 7.9)
$MnCl_2$
$ZnCl_2$ alone or
+ivermectin or
+ivermectin+ATP
Zinc
Zinc + Iver
Zinc + Iver + ATP
% of Fura-2 fluorescence (359 nm)
110
100
90
80
70
60
50
0
100
200
300
400
500
600
700
800
Time (sec)
NaCl
NMDG-Cl (pH 7.9)
ATP (100 μM) ± ivermectin (10 μM)
+ Ivermectin
-Ivermectin
$[Ca^{2+}]_i$ (nm)
260
240
220
200
180
160
140
120
100
80
60
40
20
0
5
10
15
20
25
30
Time (min)

FIG.6A

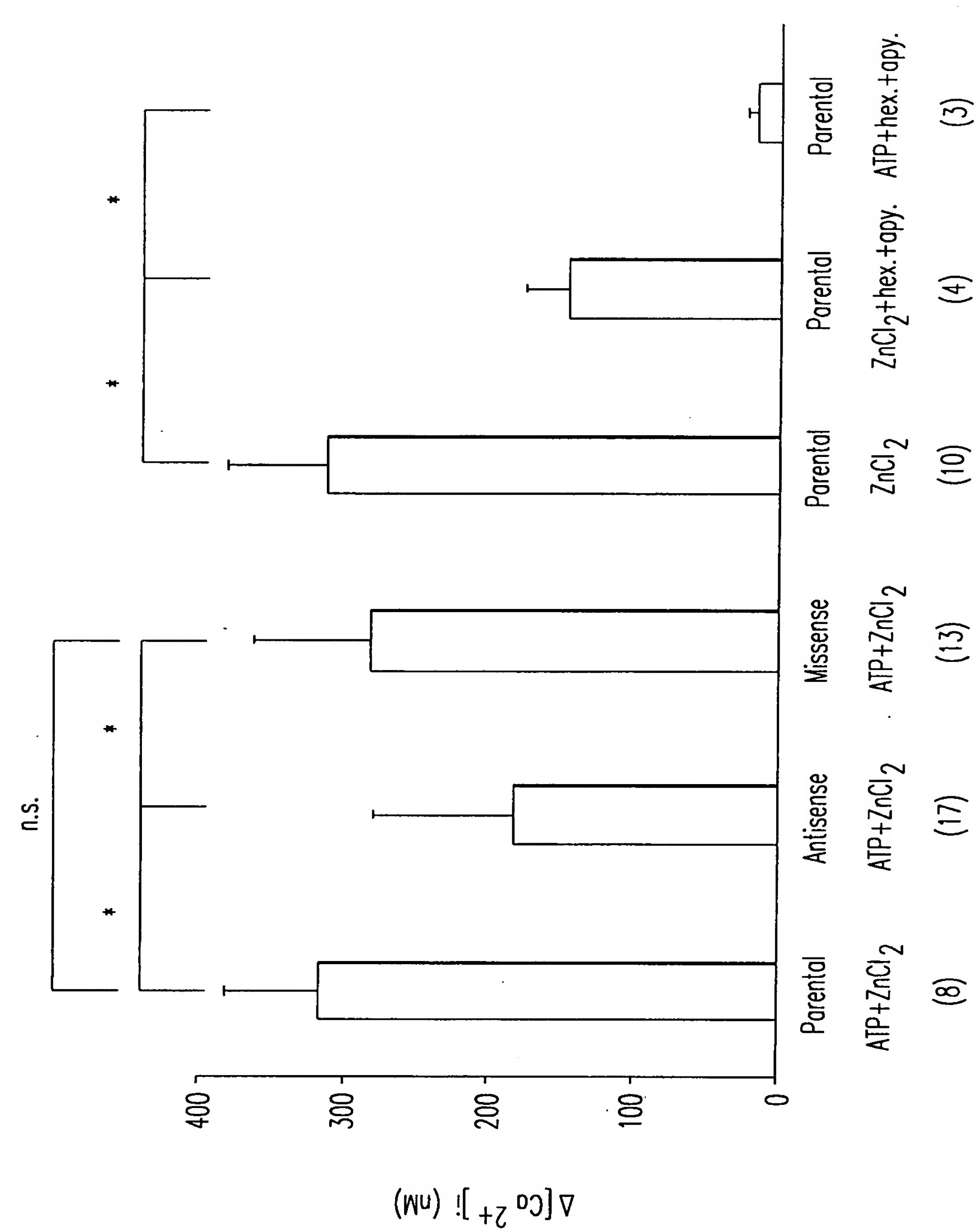

Δ[Ca 2+]i (nM)
0
100
200
300
400
n.s.
*
*
*
*
Parental
ATP+ZnCl2
(8)
Antisense
ATP+ZnCl2
(17)
Missense
ATP+ZnCl2
(13)
Parental
ZnCl2
(10)
Parental
ZnCl2+hex.+apy.
(4)
Parental
ATP+hex.+apy.
(3)

FIG.6B

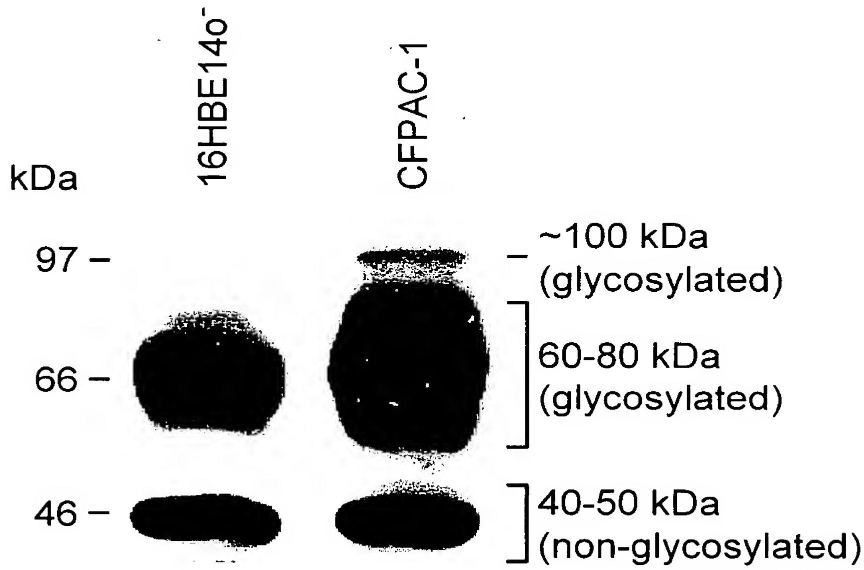

16HBE14o⁻
CFPAC-1
kDa
97 –
66 –
46 –
~100 kDa
(glycosylated)
60-80 kDa
(glycosylated)
40-50 kDa
(non-glycosylated)

FIG.7A

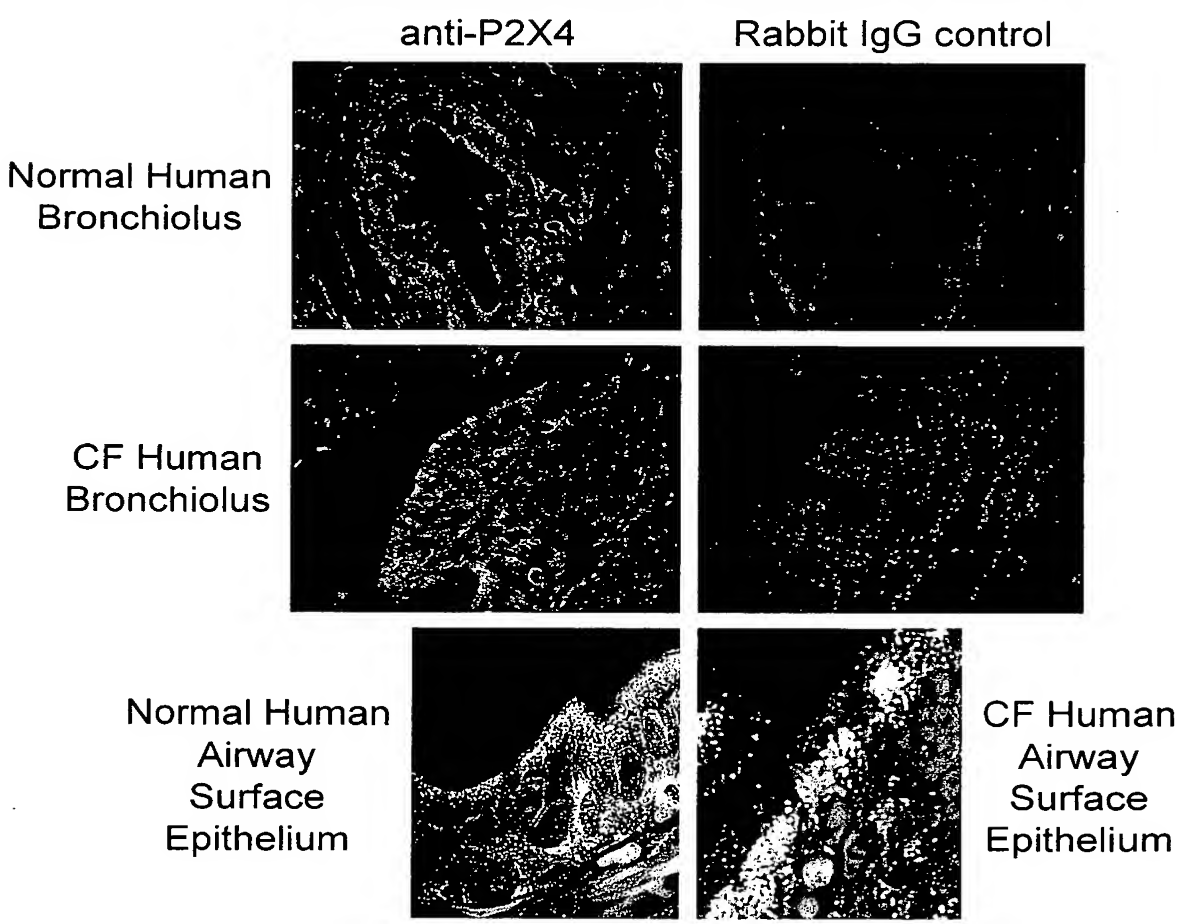

anti-P2X4
Rabbit IgG control
Normal Human Bronchiolus
CF Human Bronchiolus
Normal Human Airway Surface Epithelium
CF Human Airway Surface Epithelium

FIG.7B

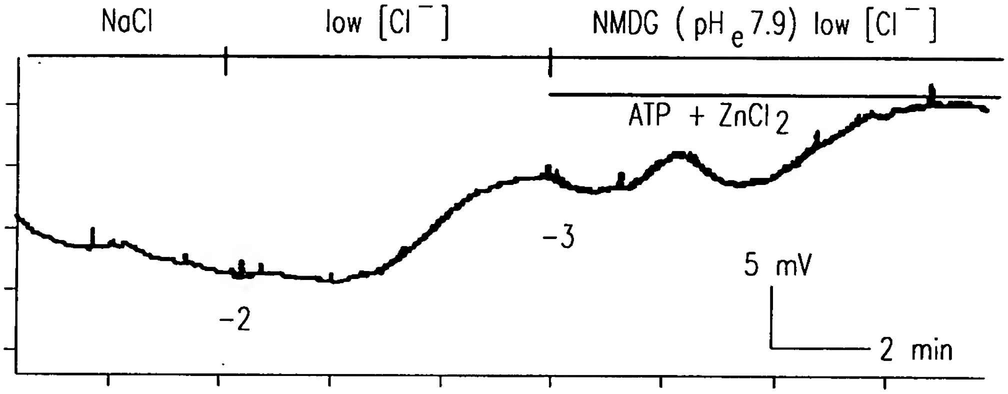


FIG.8A

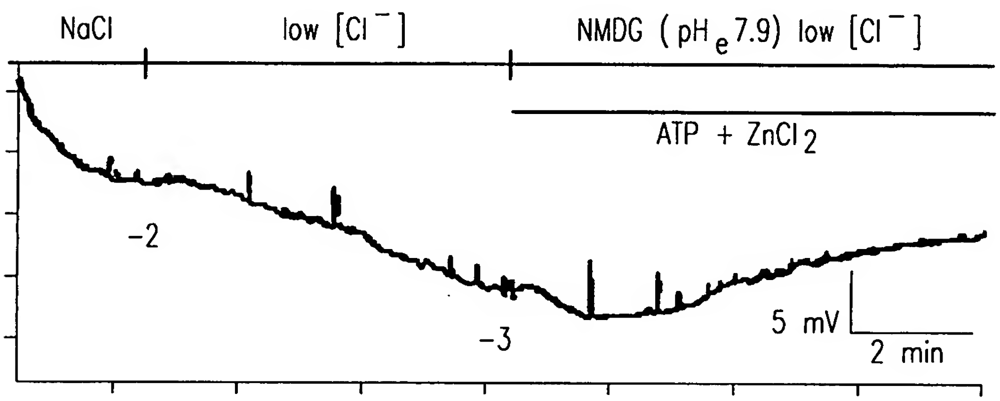


FIG.8B

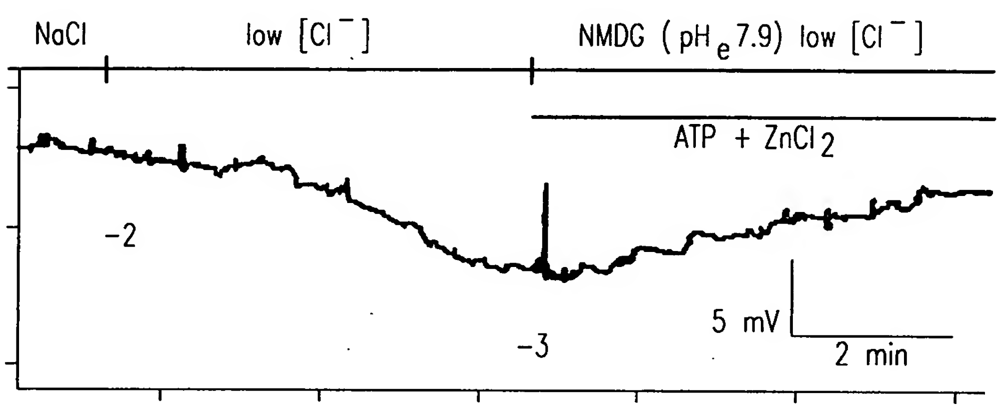


FIG.8C

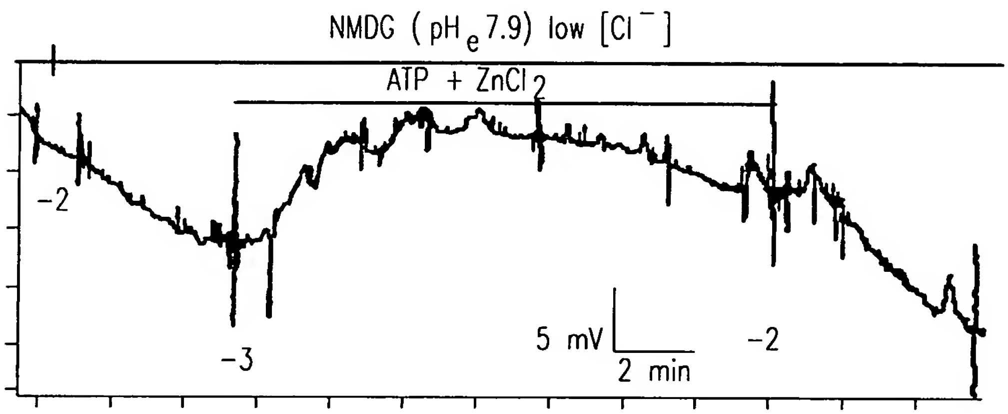

NMDG (pH e 7.9) low [Cl⁻]
ATP + ZnCl2
-2
-3
5 mV
2 min
-2

FIG.8D

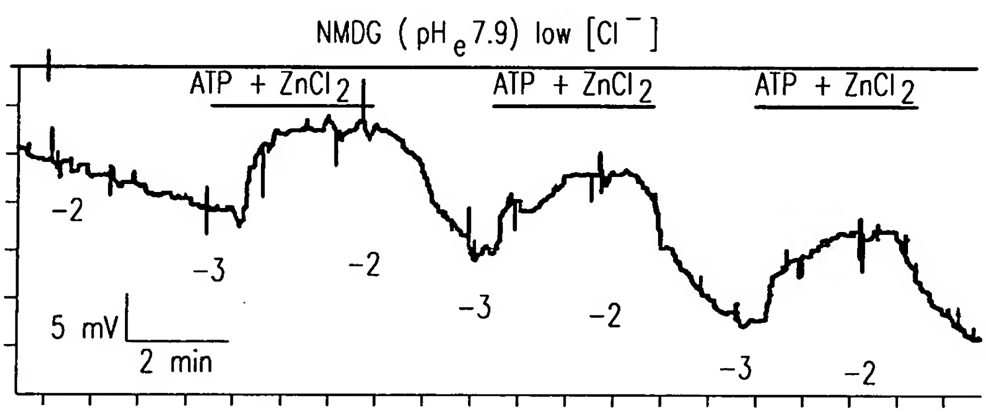

NMDG (pH e 7.9) low [Cl⁻]
ATP + ZnCl2
ATP + ZnCl2
ATP + ZnCl2
-2
-3
-2
-3
-2
-3
-2
5 mV
2 min

FIG.8E

Transepithelial Nasal Potential Difference Values of Control, Δ508 CF and Bitransgenic CF Mice

	Control Cftr(+/−)	n	CF Cftr(ΔF508/ΔF508)	n	Bitransgenic CF Cftr(−/−)	n
Starting point	−18.7 ± 6.5	19	$-26.3 \pm 7.2^{*}$	11	$-26.1 \pm 3.8^{*}$	14
Low $[Cl^-]_e$ (Na^+; pH:7.3)	−5.5 ± 1.5	8	$+3.7 \pm 1.6^{*}$	3	$+4.8 \pm 2.5^{*}$	7
ATP + $ZnCl_2$ (NMDG; pH:7.9)	−4.7 ± 1.8	6	−4.0 ± 2.0	3	−3.8 ± 2.0	12
Low $[Cl^-]_e$ (Na^+; pH:7.9)	−4.8 ± 2.0	6	$+5.4 \pm 2.8^{*}$	7	$+6.7 \pm 4.0^{*}$	3
ATP + $ZnCl_2$ (NMDG; pH:7.9)	−6.0 ± 1.4	2	$-9.4 \pm 1.6^{*\#}$	8	$-9.7 \pm 3.1^{*\&}$	3
Low $[Cl^-]_e$ (NMDG; pH:7.9)	−4.8 ± 3.3	5			$+5.8 \pm 1.9^{*}$	4
ATP + $ZnCl_2$ (NMDG; pH:7.9)	−5.7 ± 1.2	3			$-10.2 \pm 1.3^{*\&}$	6
ATP alone (NMDG; pH:7.9)					$-2.3 \pm 1.0^{\S}$	4
Low $[Cl^-]_e$ (NMDG; no added Ca^{2+}; pH:7.9)	−7.3 ± 0.6	3			$+6.0 \pm 0.8^{*}$	4
ATP + $ZnCl_2$ (NMDG; no added Ca^{2+}; pH:7.9)	$-1.3 \pm 0.6^{\$}$	3			$-2.0 \pm 1.2^{\$}$	4

FIG.8F

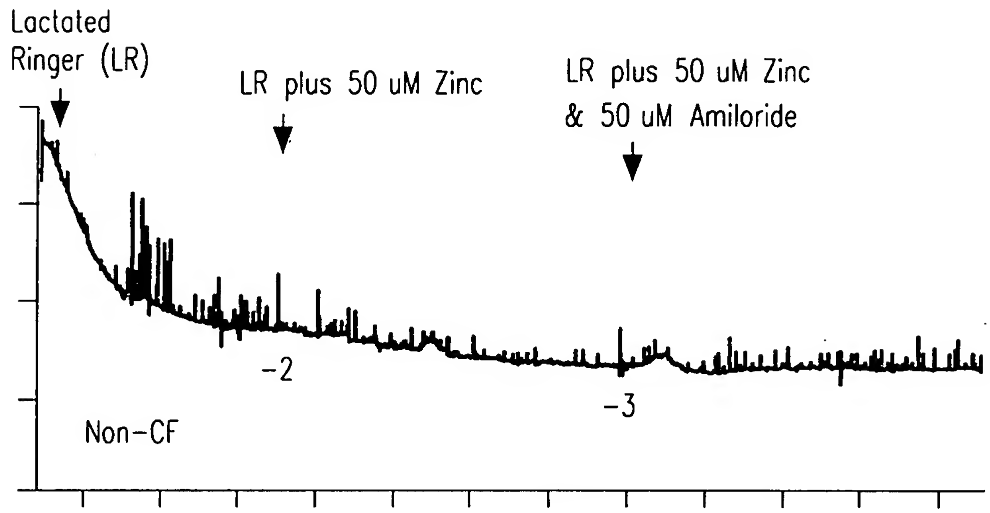

Lactated
Ringer (LR)
LR plus 50 uM Zinc
LR plus 50 uM Zinc
& 50 uM Amiloride
-2
-3
Non-CF

FIG.9A

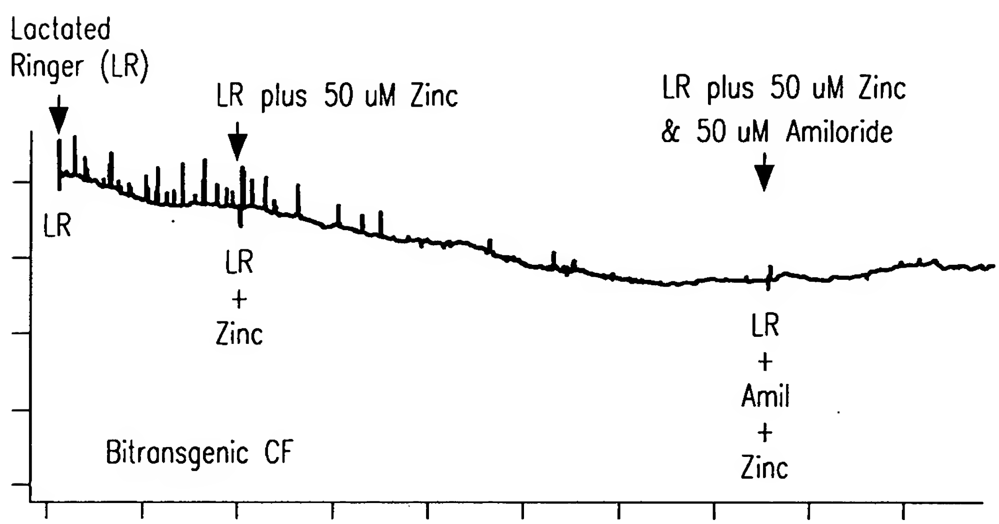

Lactated
Ringer (LR)
LR plus 50 uM Zinc
LR plus 50 uM Zinc
& 50 uM Amiloride
LR
LR
+
Zinc
LR
+
Amil
+
Zinc
Bitransgenic CF

FIG.9B

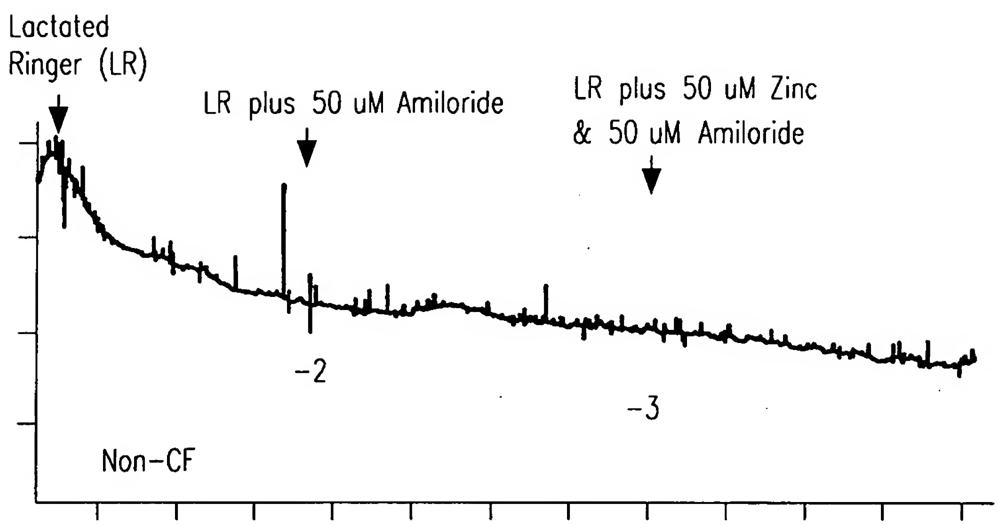

Lactated
Ringer (LR)
LR plus 50 uM Amiloride
LR plus 50 uM Zinc
& 50 uM Amiloride
-2
-3
Non-CF

FIG.9C

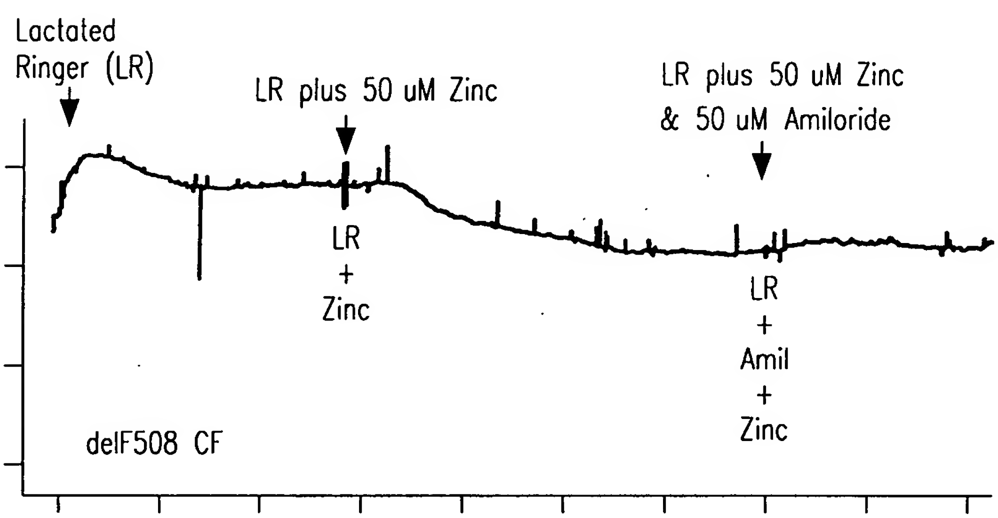

Lactated
Ringer (LR)
LR plus 50 uM Zinc
LR plus 50 uM Zinc
& 50 uM Amiloride
LR
+
Zinc
LR
+
Amil
+
Zinc
delF508 CF

FIG.9D

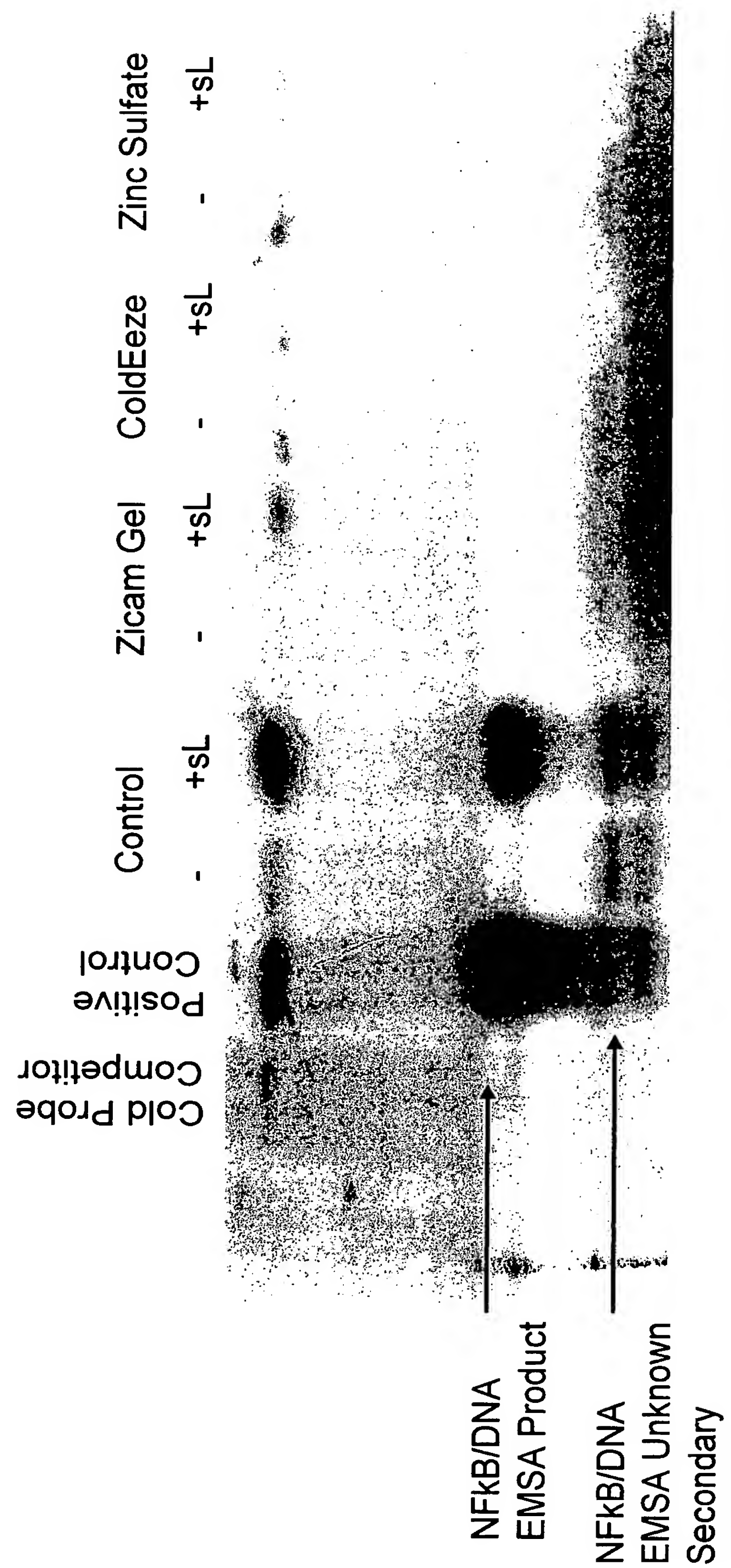

Cold Probe Competitor
Positive Control
Control
- +sL
Zicam Gel
- +sL
ColdEeze
- +sL
Zinc Sulfate
- +sL
NFkB/DNA EMSA Product
NFkB/DNA EMSA Unknown Secondary Product

FIG.10A

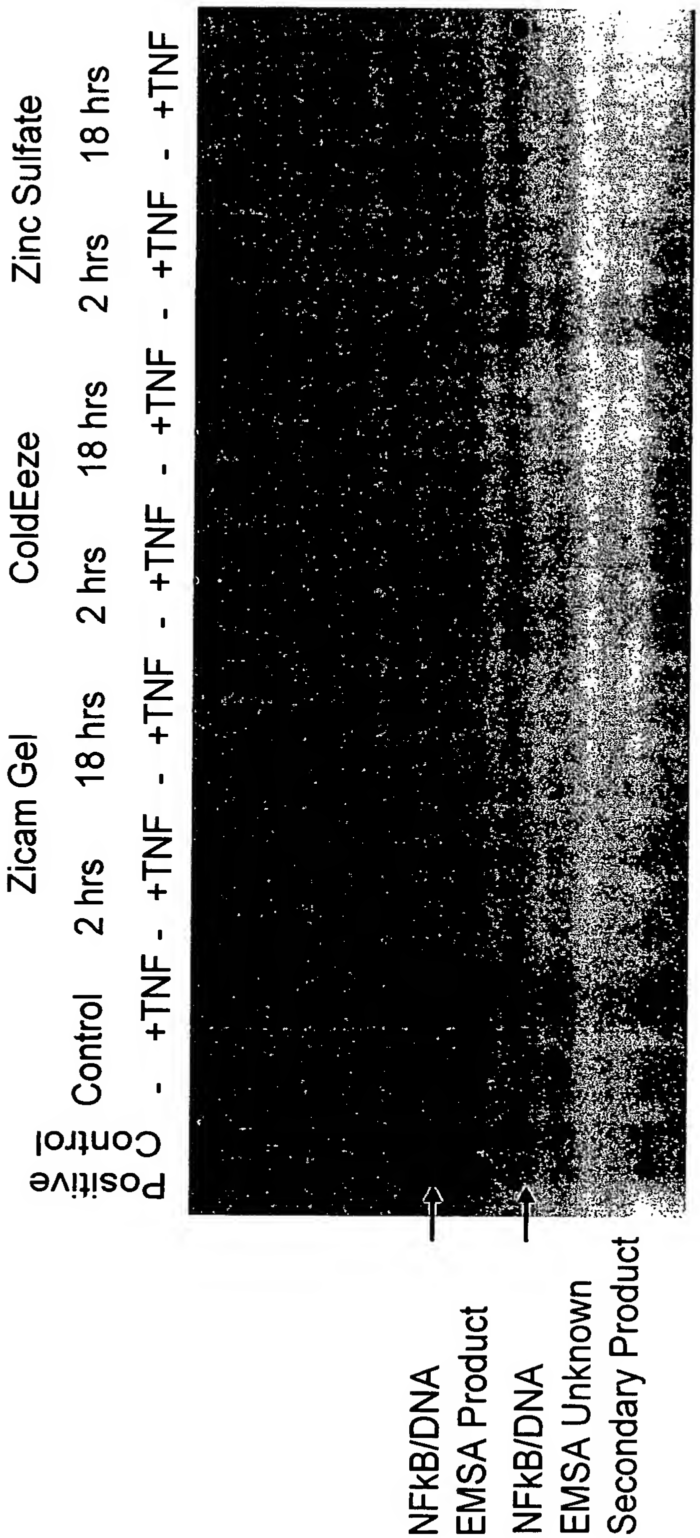

Positive Control
Control
Zicam Gel
ColdEeze
Zinc Sulfate
2 hrs
18 hrs
2 hrs
18 hrs
2 hrs
18 hrs
- +TNF - +TNF - +TNF - +TNF - +TNF - +TNF - +TNF
NFkB/DNA EMSA Product
NFkB/DNA EMSA Unknown Secondary Product

FIG.10B

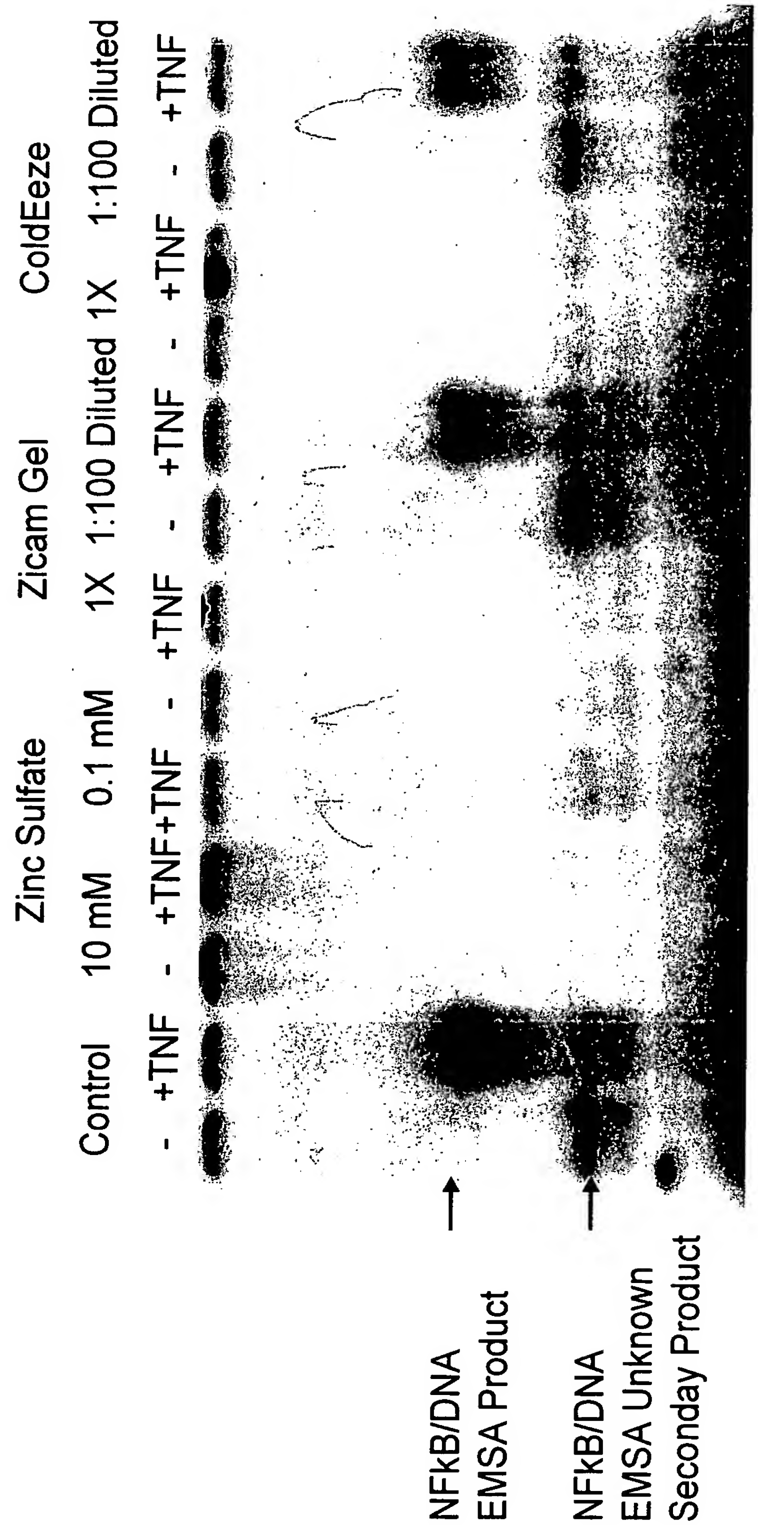

Control
Zinc Sulfate
Zicam Gel
ColdEeze
10 mM
0.1 mM
1X
1:100 Diluted
1X
1:100 Diluted
- +TNF - +TNF+TNF - +TNF - +TNF - +TNF - +TNF - +TNF
NFkB/DNA
EMSA Product
NFkB/DNA
EMSA Unknown
Seconday Product

FIG.10C

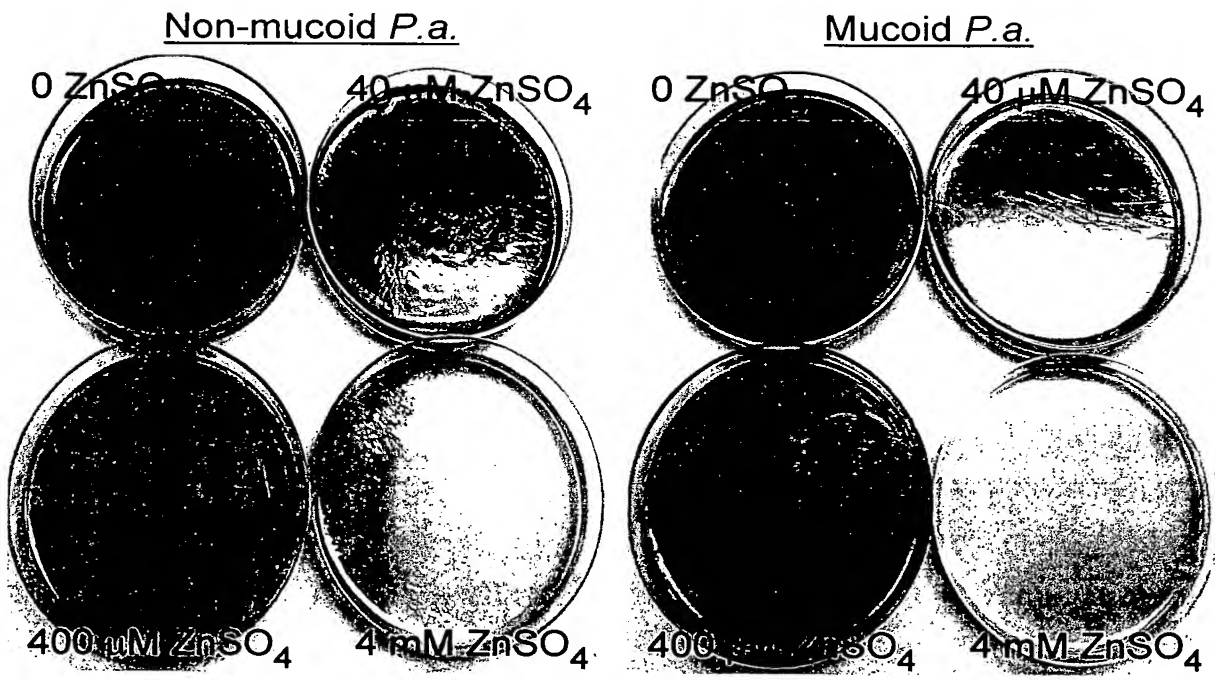


FIG.11A

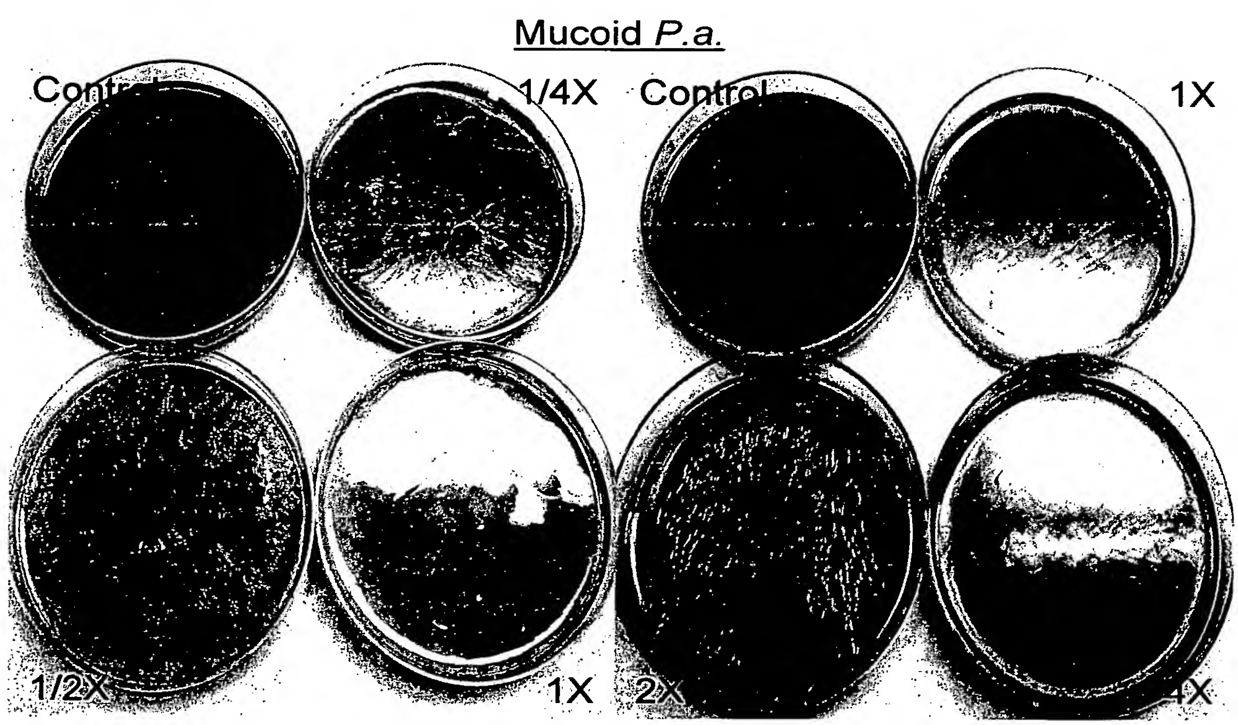
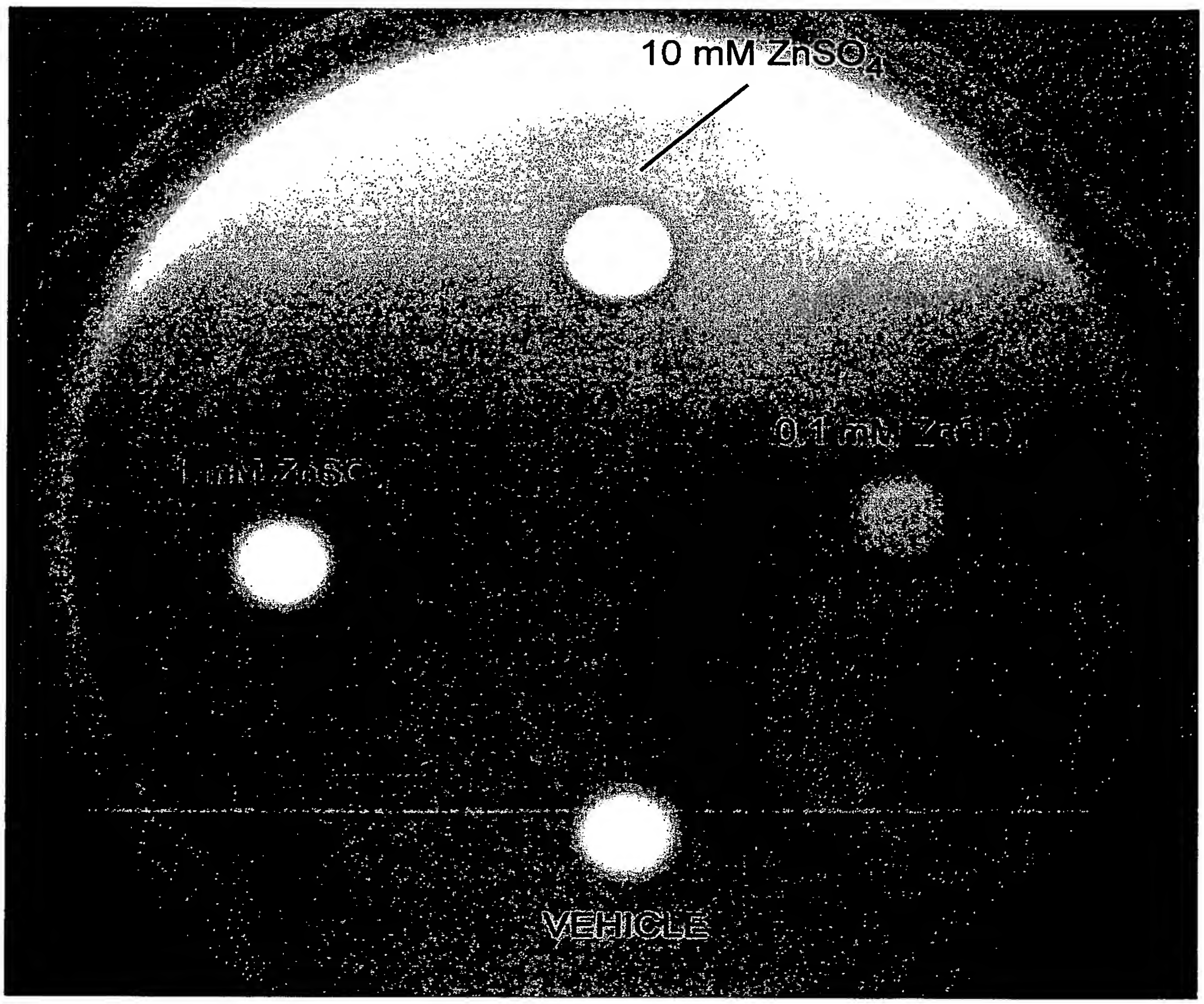


FIG.11B



E. coli.

FIG. 11C

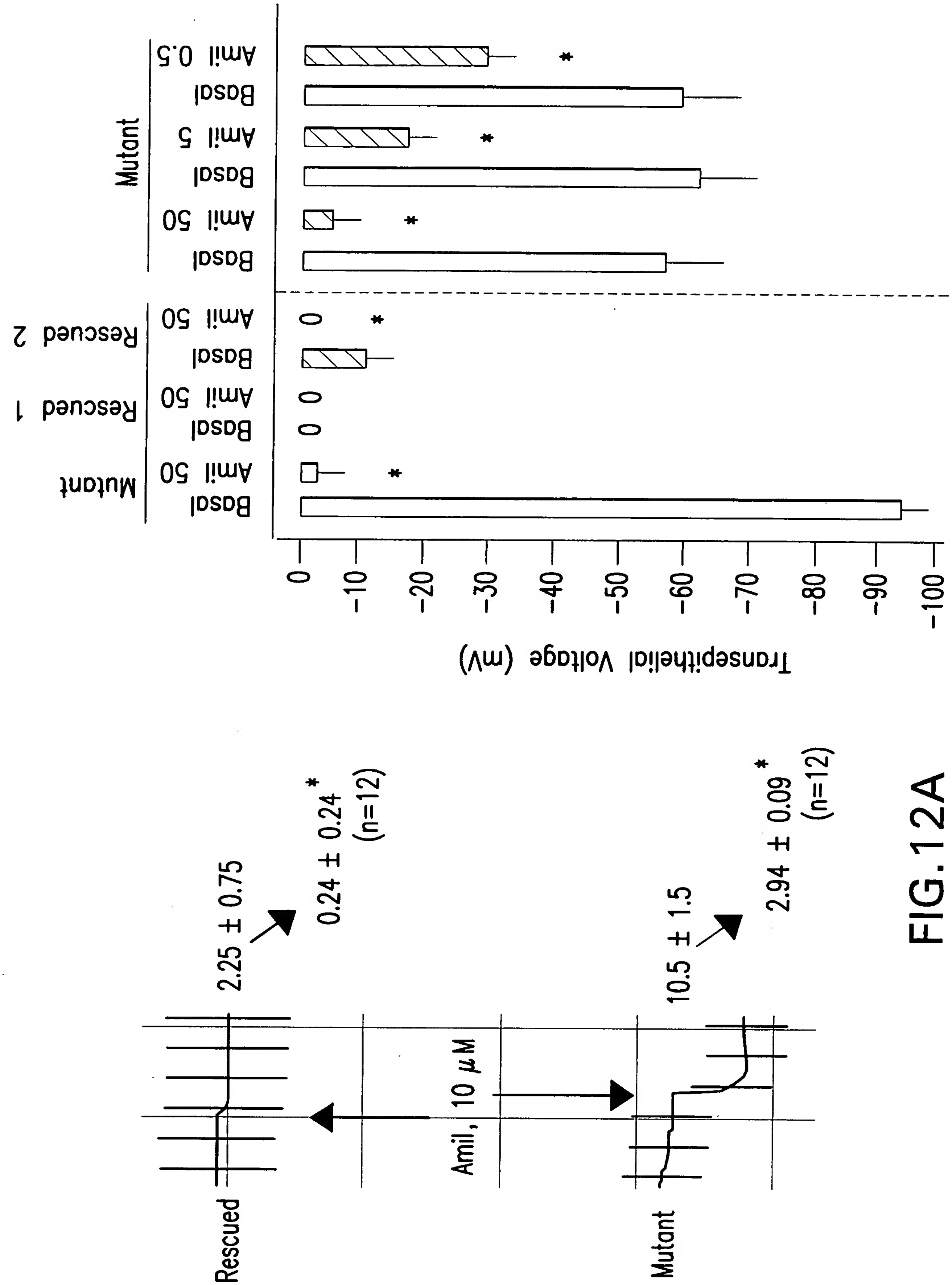
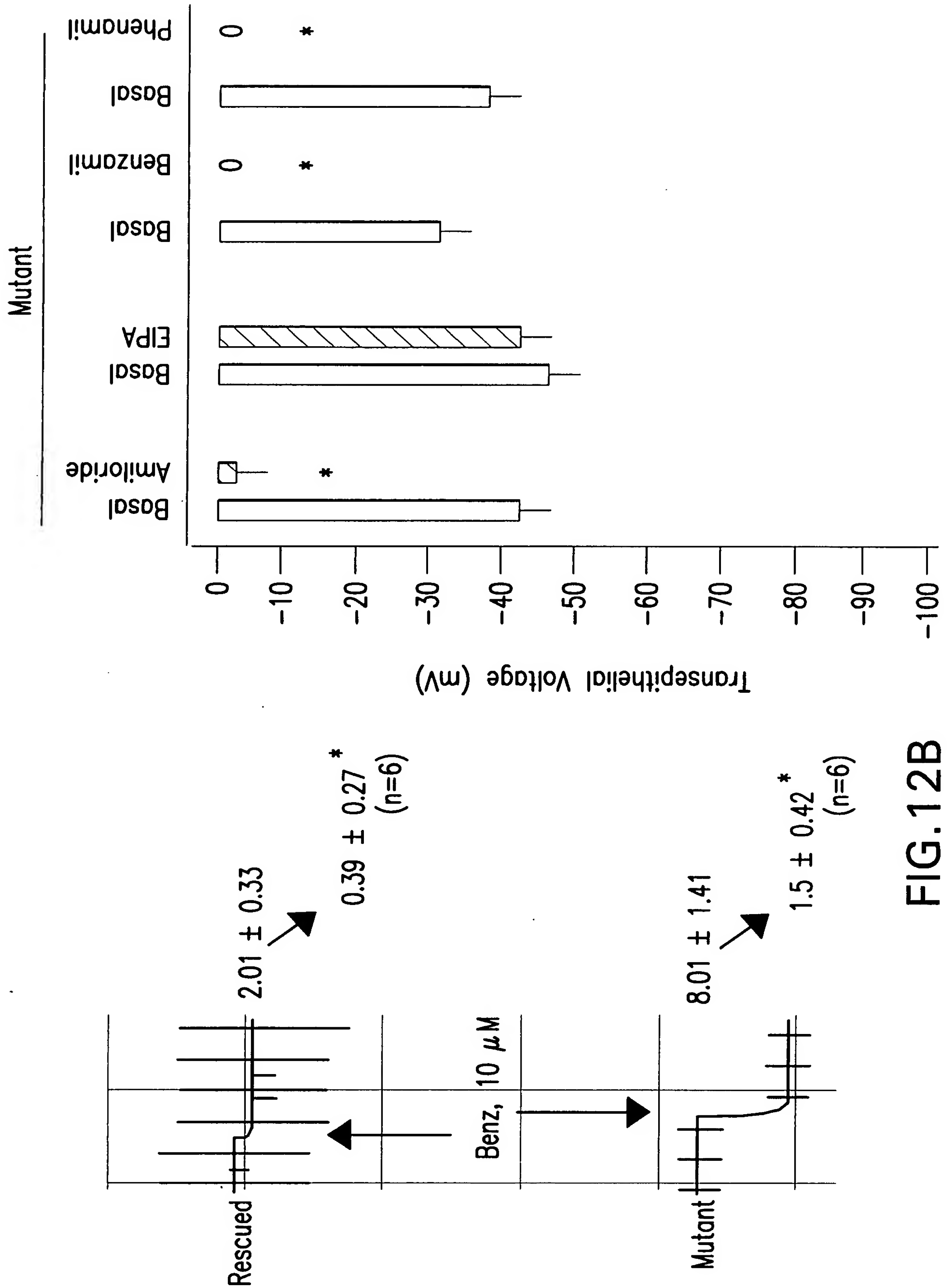

Mutant
Rescued 1
Rescued 2
Mutant
Basal
Amil 50
Basal
Amil 50
Basal
Amil 50
Basal
Amil 50
Basal
Amil 5
Basal
Amil 0.5
0
0
0
0
*
Transepithelial Voltage (mV)
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
Rescued
Mutant
Amil, 10 μM
2.25 ± 0.75
0.24 ± 0.24*
(n=12)
10.5 ± 1.5
2.94 ± 0.09*
(n=12)

FIG.12A



Mutant
Basal
Amiloride
Basal
EIPA
Basal
Benzamil
Basal
Phenamil
0
0
*
*
*
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
Transepithelial Voltage (mV)
Rescued
Mutant
Benz, 10 μM
2.01 ± 0.33
0.39 ± 0.27*
(n=6)
8.01 ± 1.41
1.5 ± 0.42*
(n=6)

FIG.12B

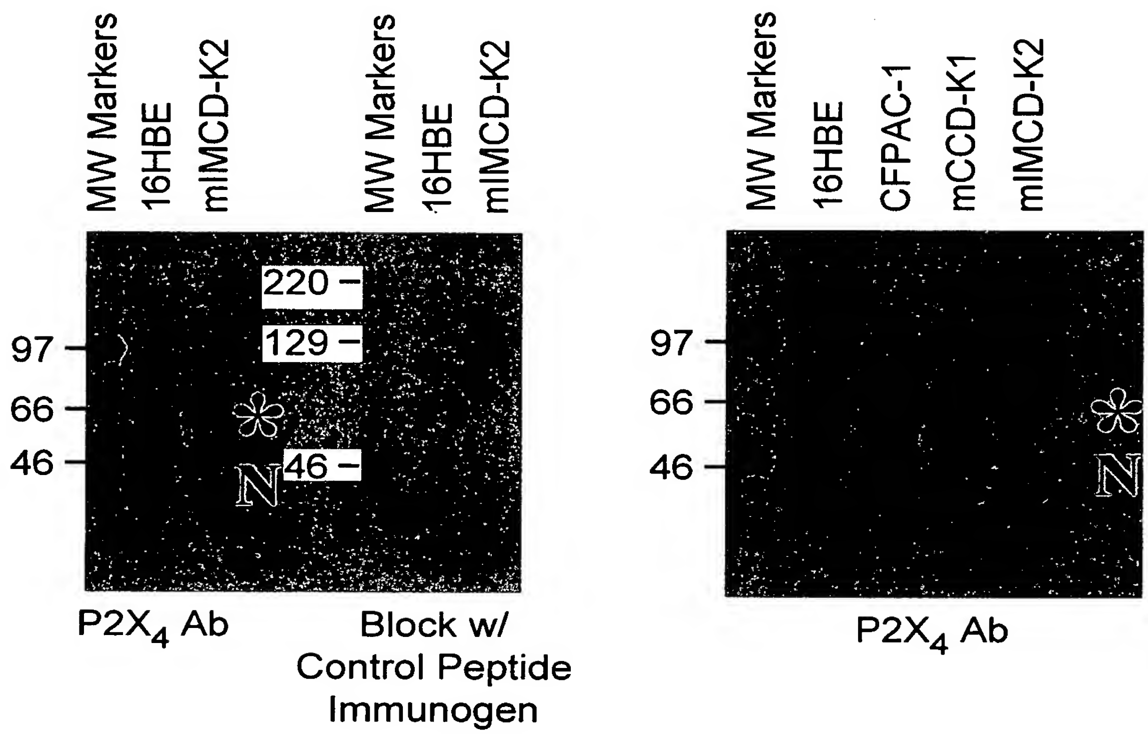

MW Markers
16HBE
mIMCD-K2
MW Markers
16HBE
mIMCD-K2
97
66
46
220
129
46
N
P2X4 Ab
Block w/ Control Peptide Immunogen
MW Markers
16HBE
CFPAC-1
mCCD-K1
mIMCD-K2
97
66
46
N
P2X4 Ab

FIG.13A

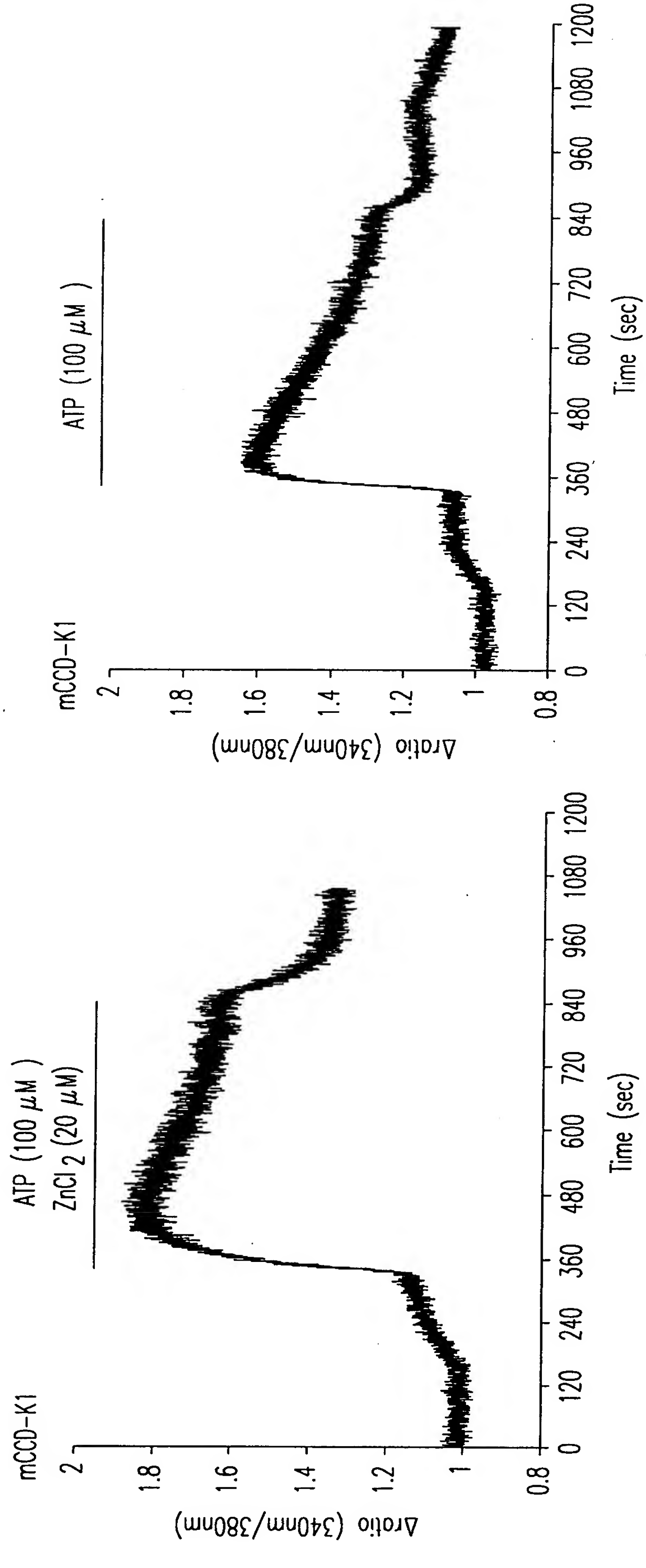

mCCD-K1
ATP (100 μM)
ZnCl2 (20 μM)
Δratio (340nm/380nm)
Time (sec)
0
120
240
360
480
600
720
840
960
1080
1200
0.8
1
1.2
1.4
1.6
1.8
2
mCCD-K1
ATP (100 μM)
Δratio (340nm/380nm)
Time (sec)

FIG.13B-1

Inventor: Schwiebert et al.
Title: Methods and Compositions for P2X Receptor Calcium Entry Channels and Other Calcium Entry Mechanisms
Docket No.: 21085.0044U3
REPLACEMENT SHEET

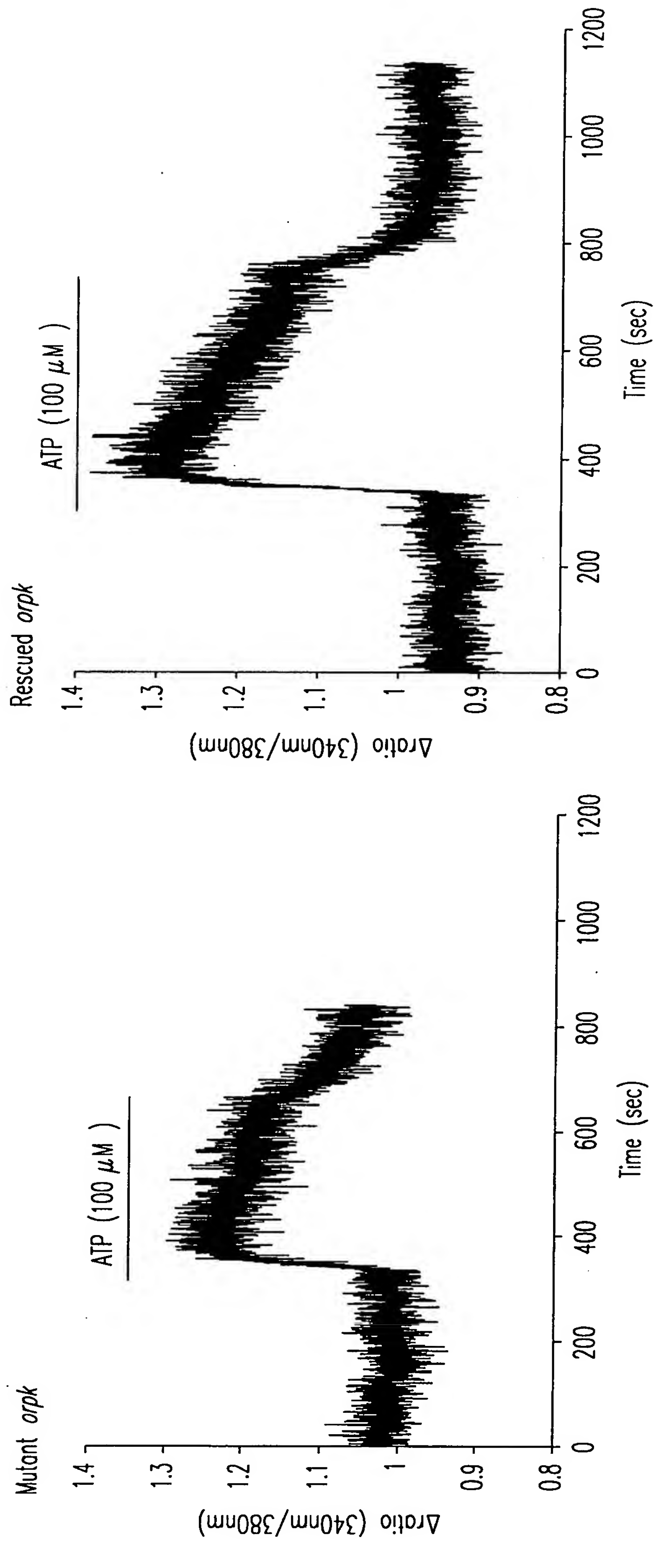


FIG.13B-2

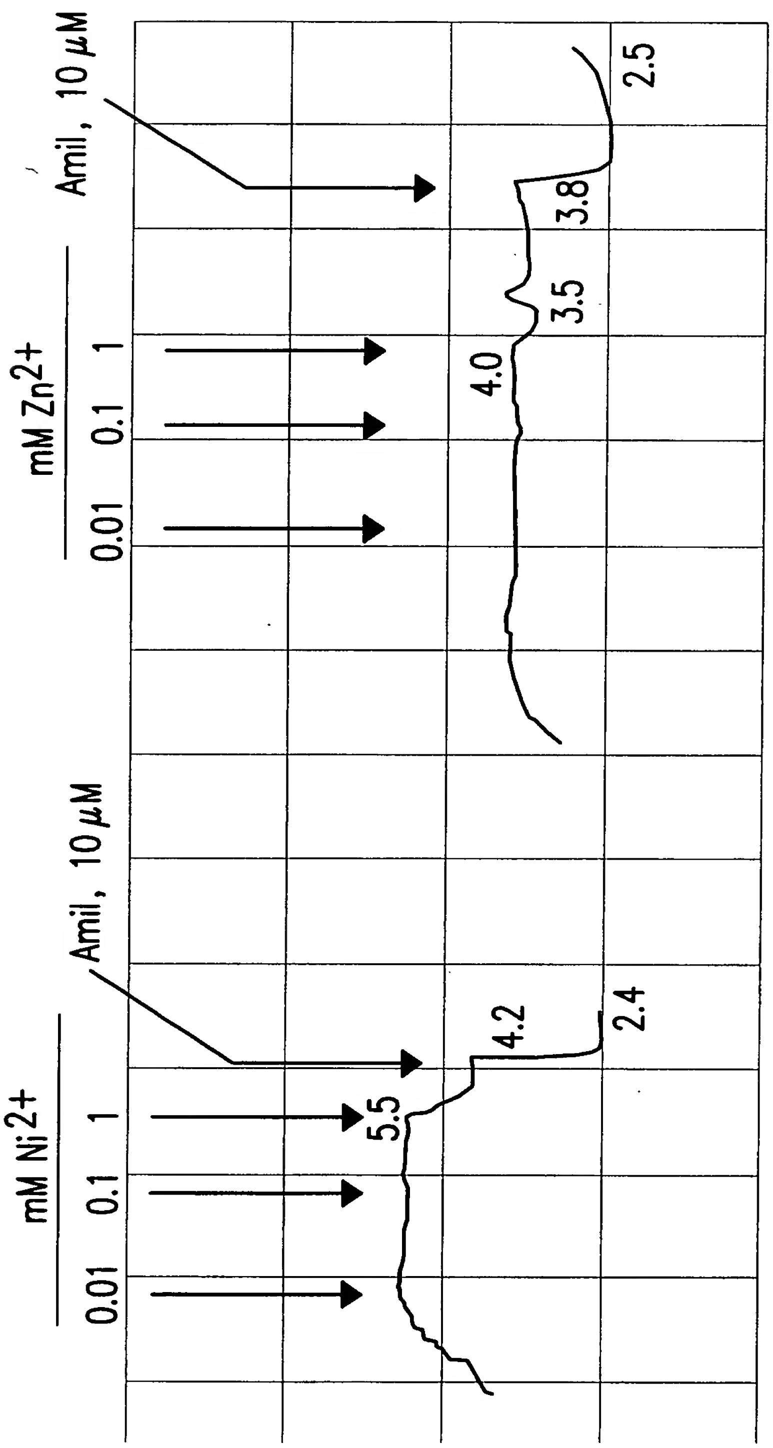

mM Ni2+
0.01
0.1
1
Amil, 10 μM
5.5
4.2
2.4
mM Zn2+
0.01
0.1
1
Amil, 10 μM
4.0
3.5
3.8
2.5

FIG.14A

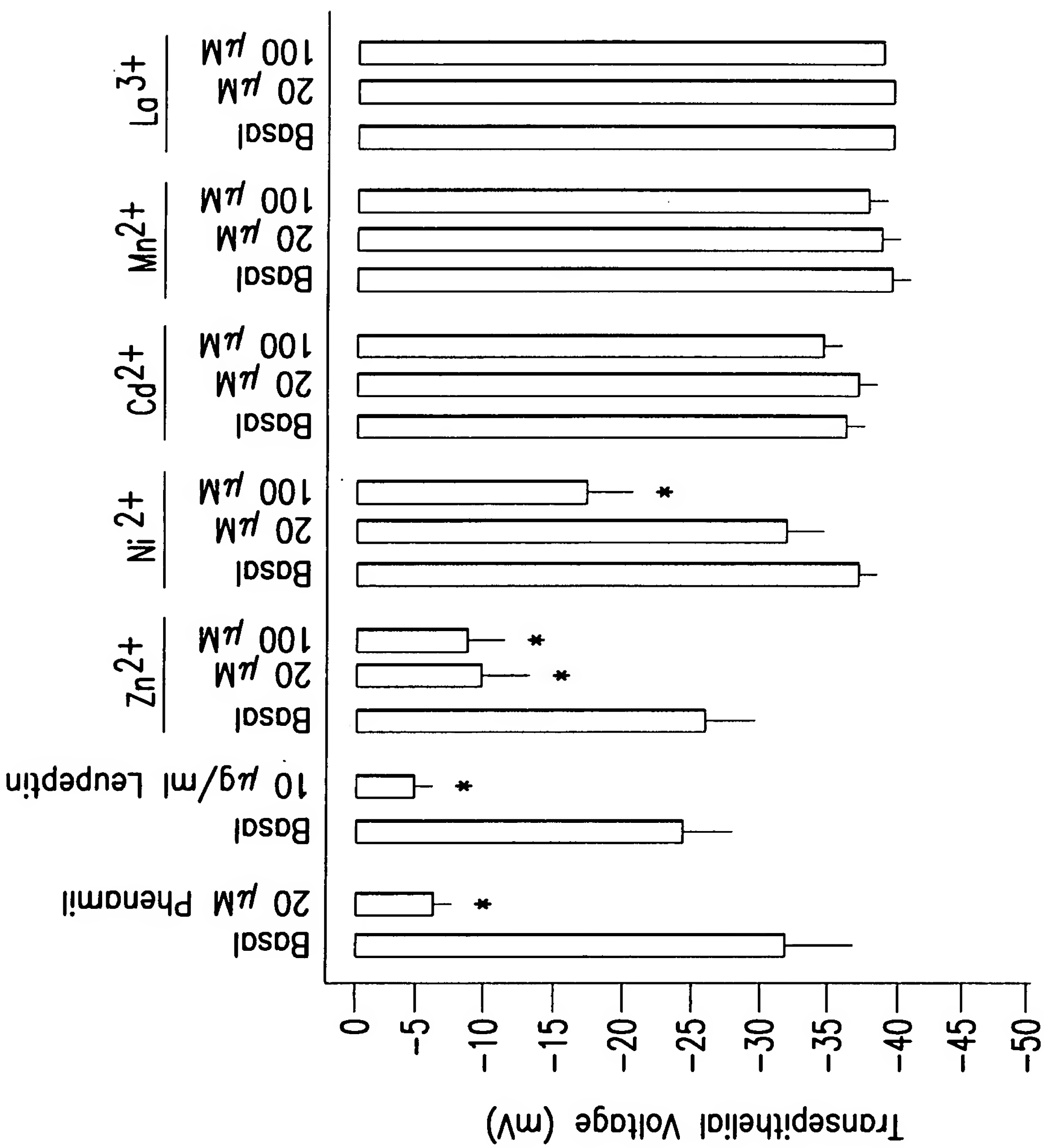
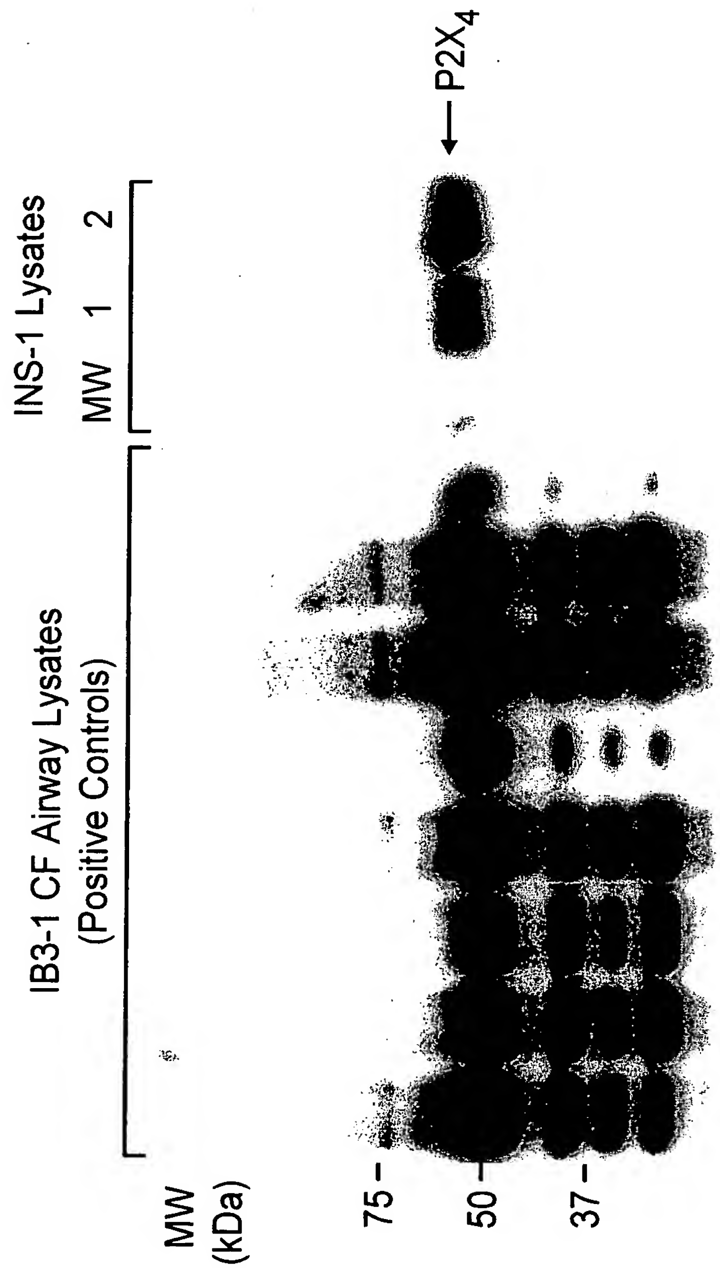

Basal
20 μM Phenamil
Basal
10 μg/ml Leupeptin
Zn2+
Basal
20 μM
100 μM
Ni 2+
Basal
20 μM
100 μM
Cd2+
Basal
20 μM
100 μM
Mn2+
Basal
20 μM
100 μM
La3+
Basal
20 μM
100 μM
*
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
Transepithelial Voltage (mV)

FIG.14B



IB3-1 CF Airway Lysates
(Positive Controls)
INS-1 Lysates
MW 1 2
P2X$_4$
MW
(kDa)
75
50
37

FIG.15A

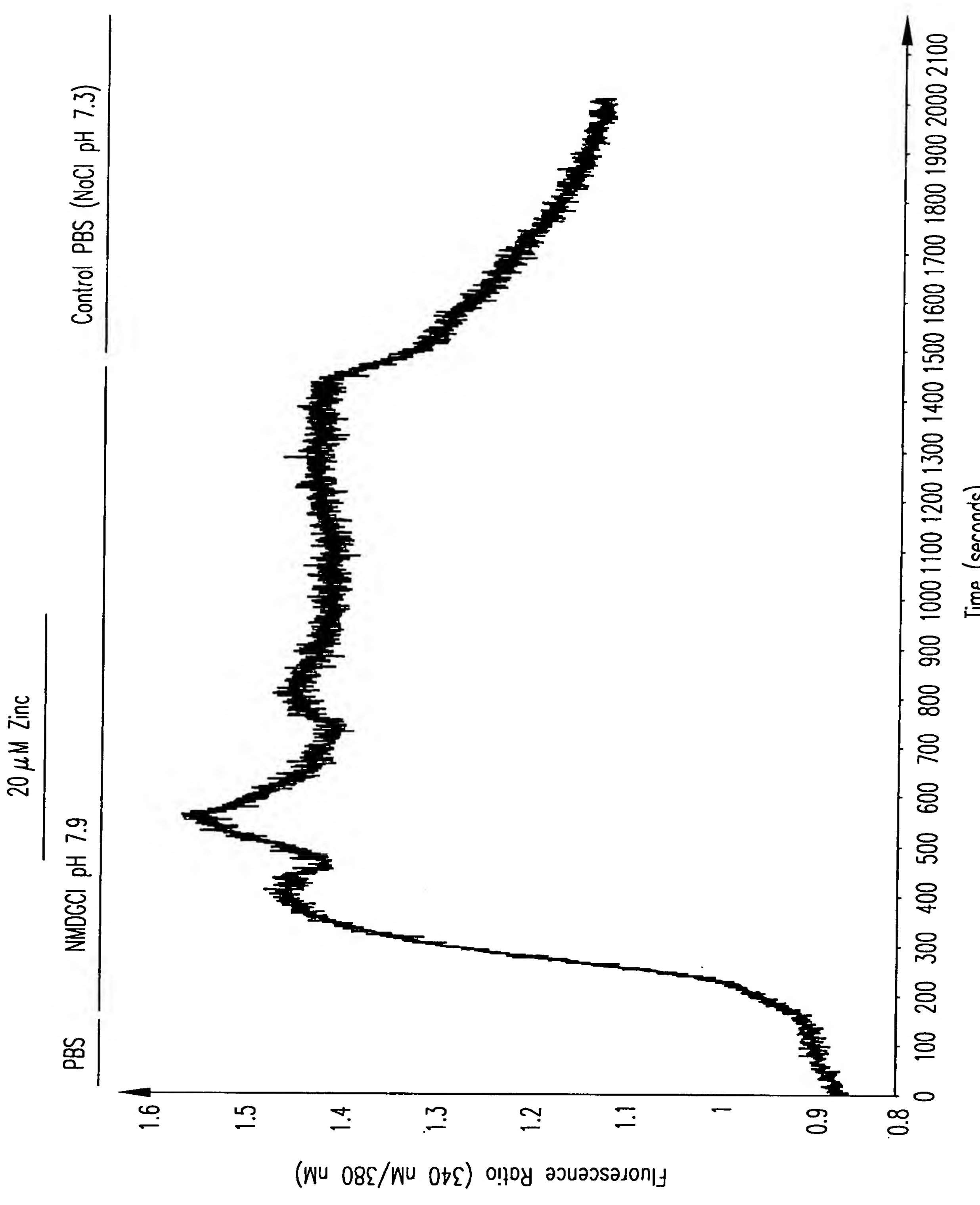

20 μM Zinc
PBS
NMDGCl pH 7.9
Control PBS (NaCl pH 7.3)
Fluorescence Ratio (340 nM/380 nM)
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
Time (seconds)

FIG.15B

Modified Saline** (pH 7.3)			Modified Saline (pH 7.3) + 15 mM Glucose		
Time	Absorbance	[Insulin]	Time	Absorbance	[Insulin]
15"	0.682 ± 0.03	~3.0 ng/ml	15"	1.070 ± 0.05	~5.0 ng/ml
15'	0.765 ± 0.04	3.25	15'	0.957 ± 0.07	4.5
30'	0.794 ± 0.06	3.5	30'	1.204 ± 0.10	5.5
60'	1.794 ± 0.09	9.0	60'	2.065 ± 0.05	11.0
120'	1.137 ± 0.05	5.0	120'	1.105 ± 0.18	5.0

*Generous gift of Dr. Chris Newgard at Duke.

**Modified saline is 0 Na (substituted fully by NMDG), 0 Mg, and 3 mM Ca.

Standard Curve

Absorbance	[Insulin]
0.248	0.0
0.226	0.2 ng/ml
0.280	0.5 ng/ml
0.377	1.0 ng/ml
0.559	2.0 ng/ml
1.10	5.0 ng/ml
1.91	10.0 ng/ml
~3.0	~20 ng.ml

FIG. 16A

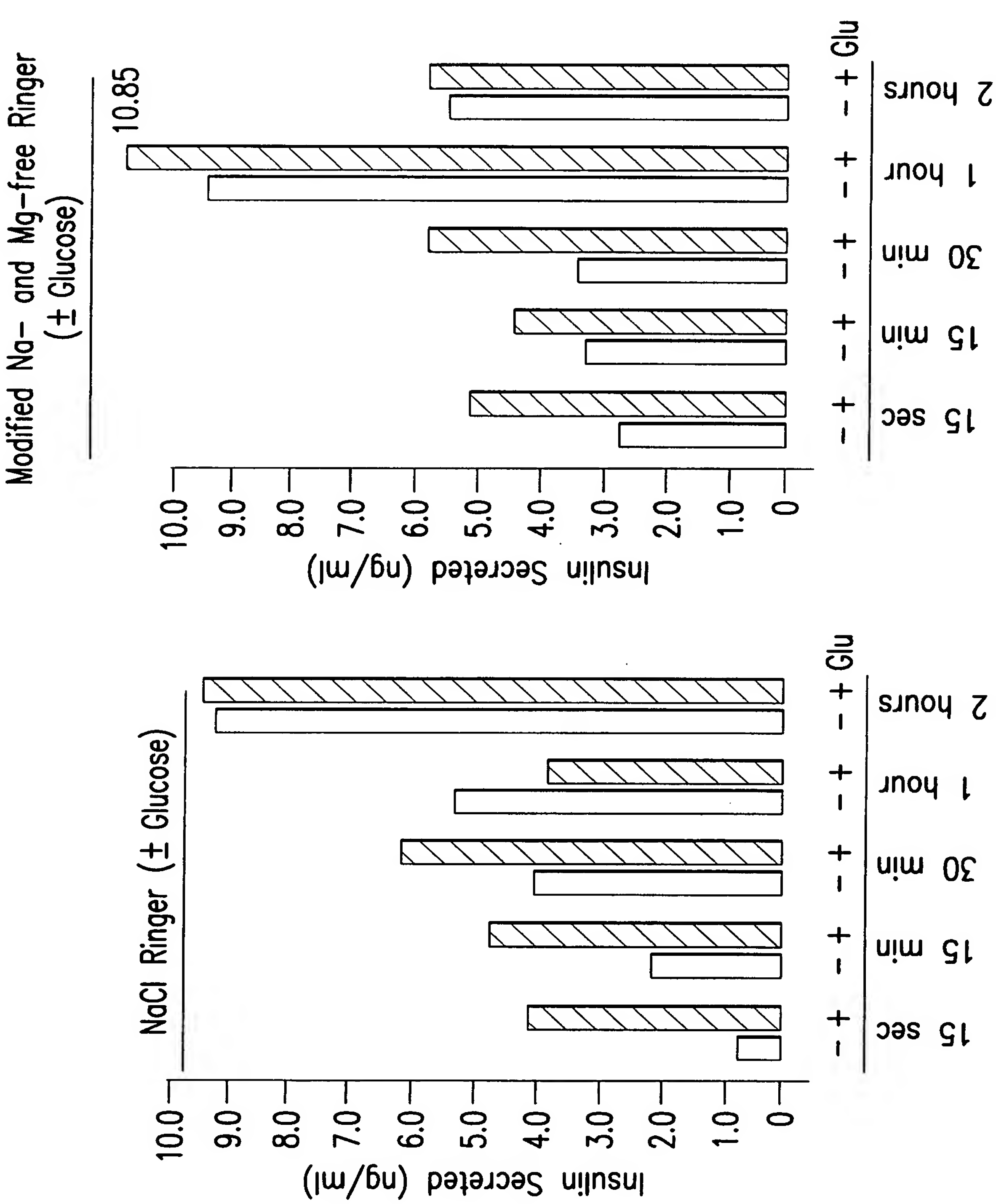
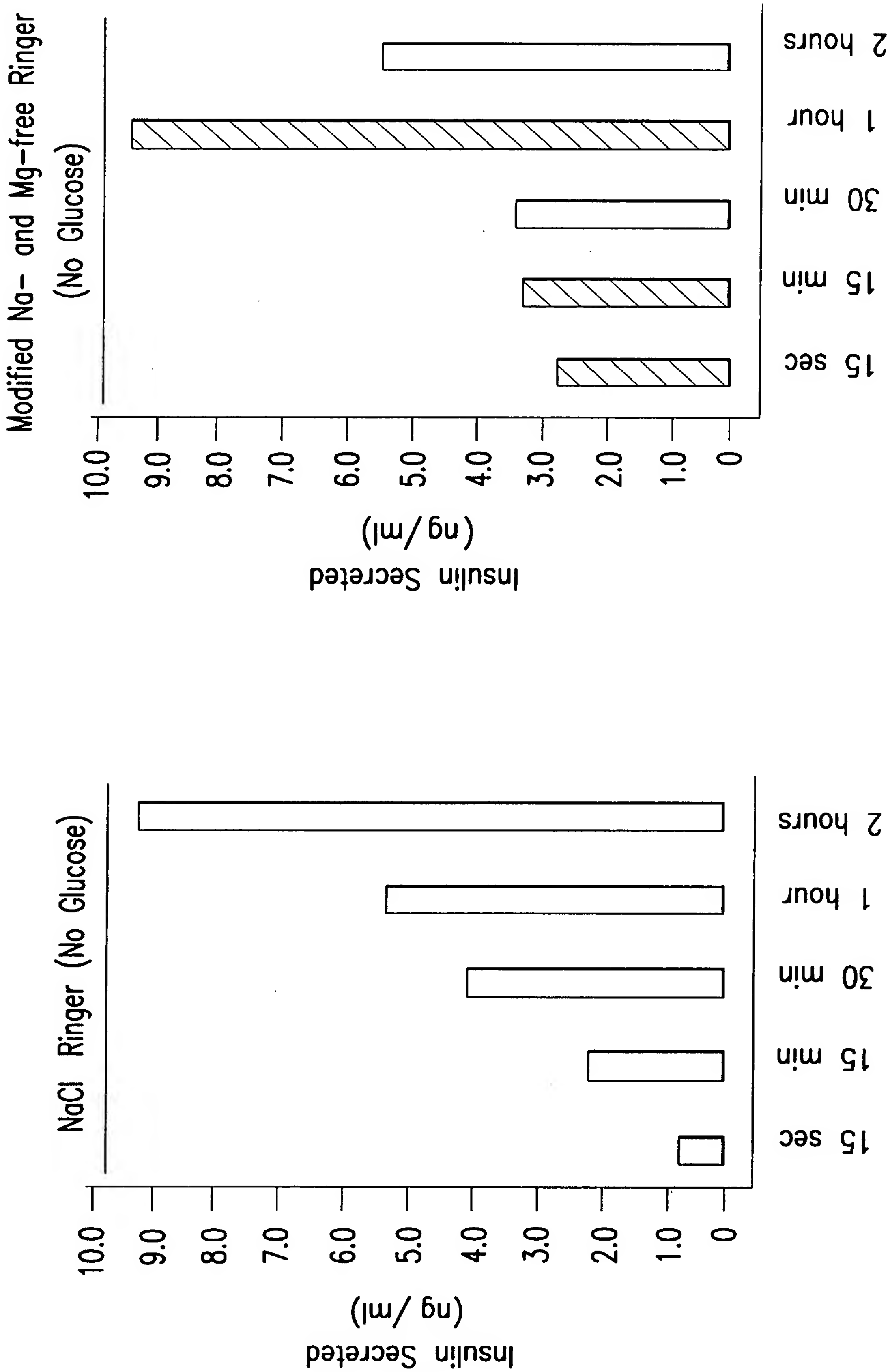

Modified Na- and Mg-free Ringer
(± Glucose)
10.85
Insulin Secreted (ng/ml)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
- + - + - + - + - + Glu
15 sec
15 min
30 min
1 hour
2 hours
NaCl Ringer (± Glucose)
Insulin Secreted (ng/ml)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
- + - + - + - + - + Glu
15 sec
15 min
30 min
1 hour
2 hours

FIG.16B



Modified Na- and Mg-free Ringer (No Glucose)
NaCl Ringer (No Glucose)
Insulin Secreted (ng/ml)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
15 sec
15 min
30 min
1 hour
2 hours

FIG.17A

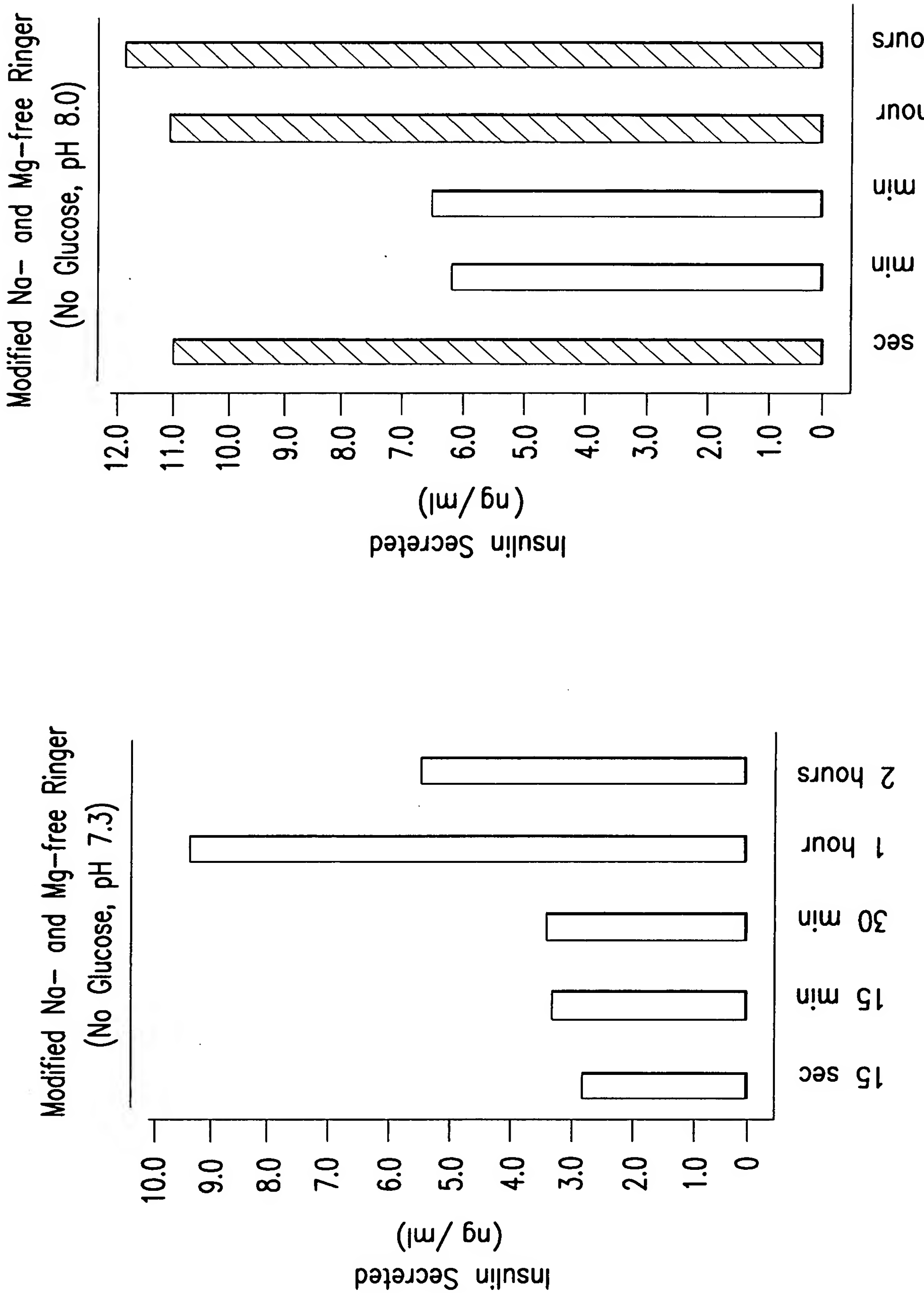

Modified Na– and Mg–free Ringer
(No Glucose, pH 8.0)
Insulin Secreted
(ng/ml)
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
15 sec
15 min
30 min
1 hour
2 hours
Modified Na– and Mg–free Ringer
(No Glucose, pH 7.3)
Insulin Secreted
(ng/ml)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
15 sec
15 min
30 min
1 hour
2 hours

FIG.17B

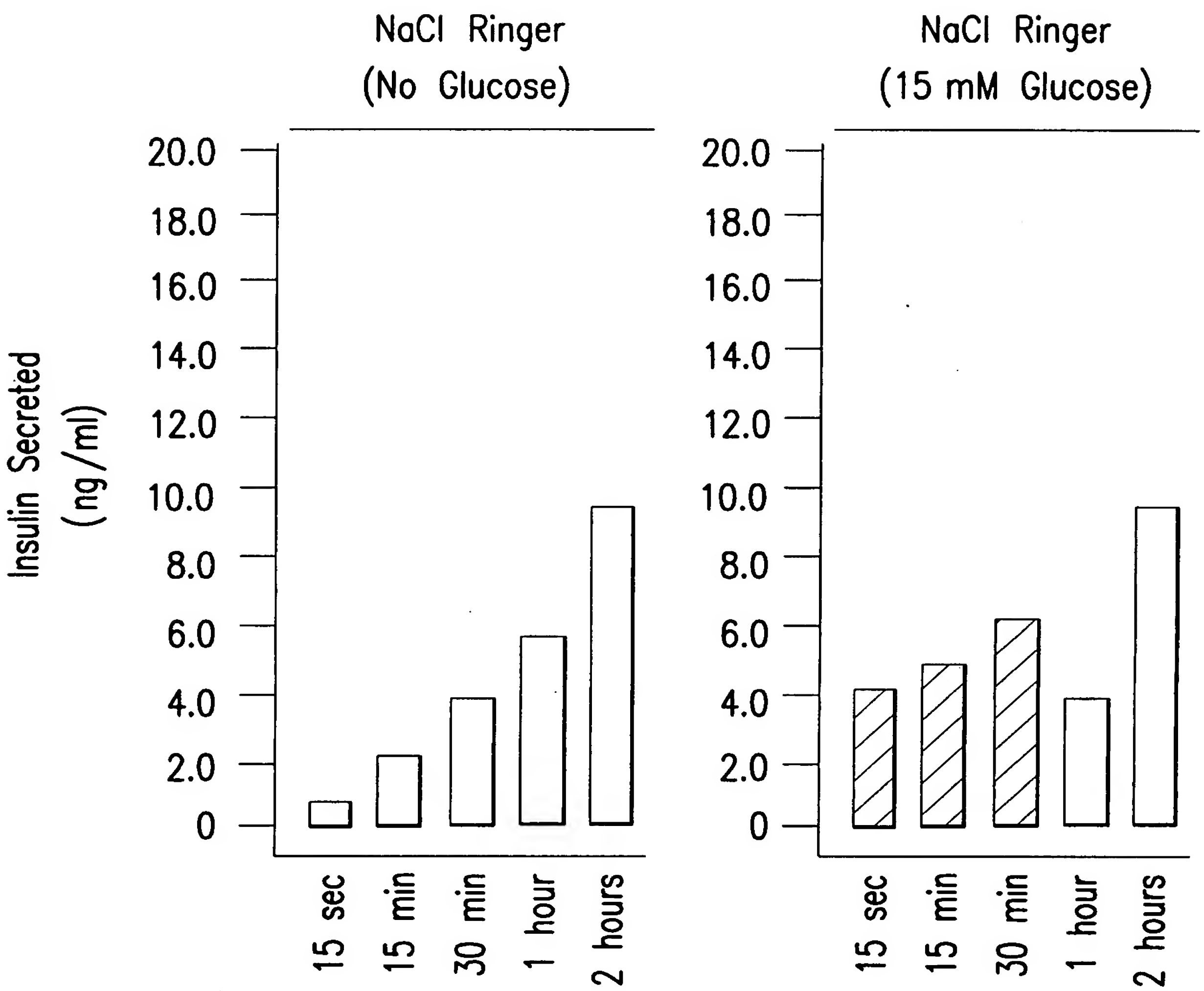

NaCl Ringer
(No Glucose)
NaCl Ringer
(15 mM Glucose)
Insulin Secreted
(ng/ml)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0
15 sec
15 min
30 min
1 hour
2 hours

FIG.18A

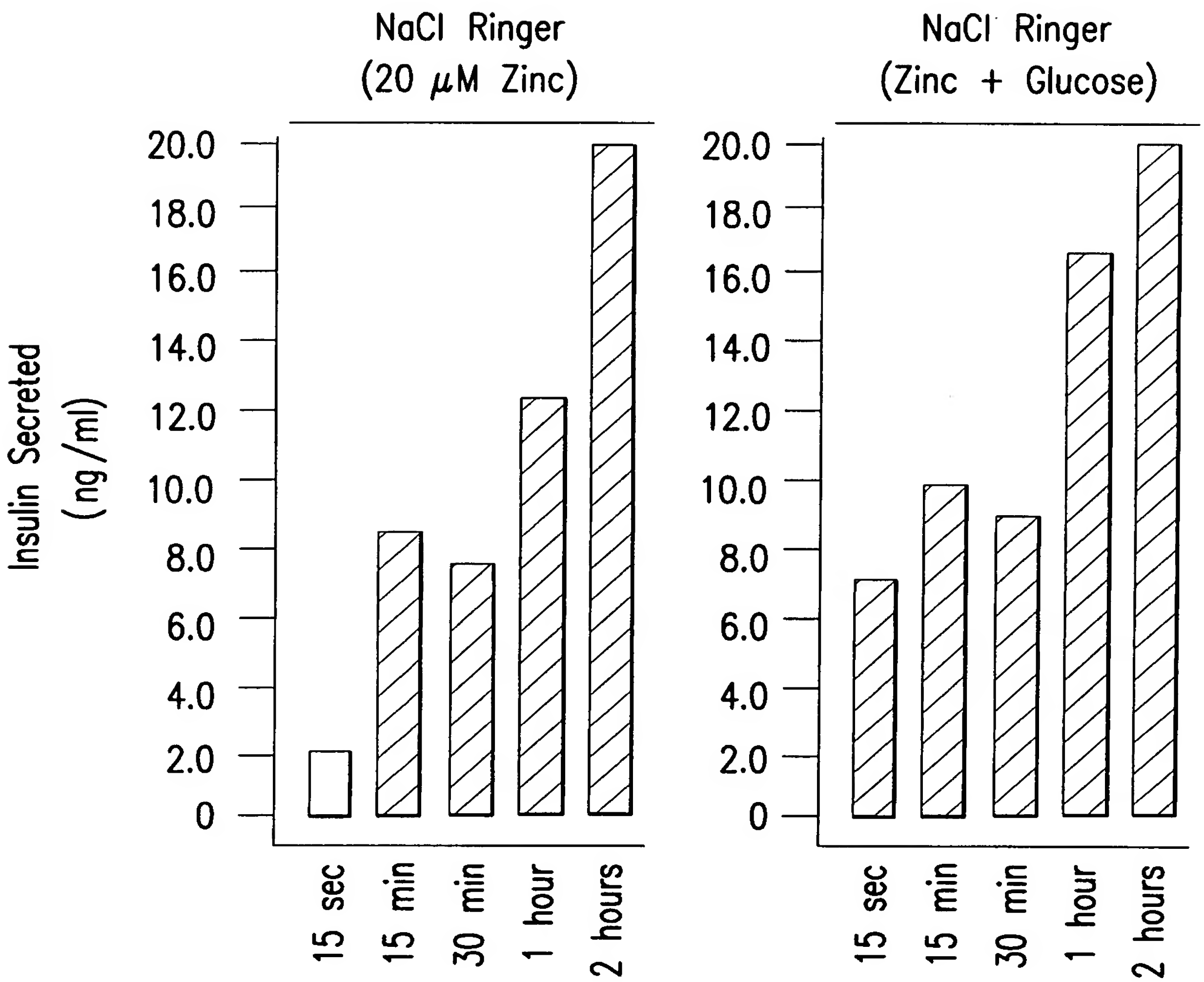

NaCl Ringer
(20 μM Zinc)
NaCl Ringer
(Zinc + Glucose)
Insulin Secreted
(ng/ml)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0
15 sec
15 min
30 min
1 hour
2 hours

FIG.18B

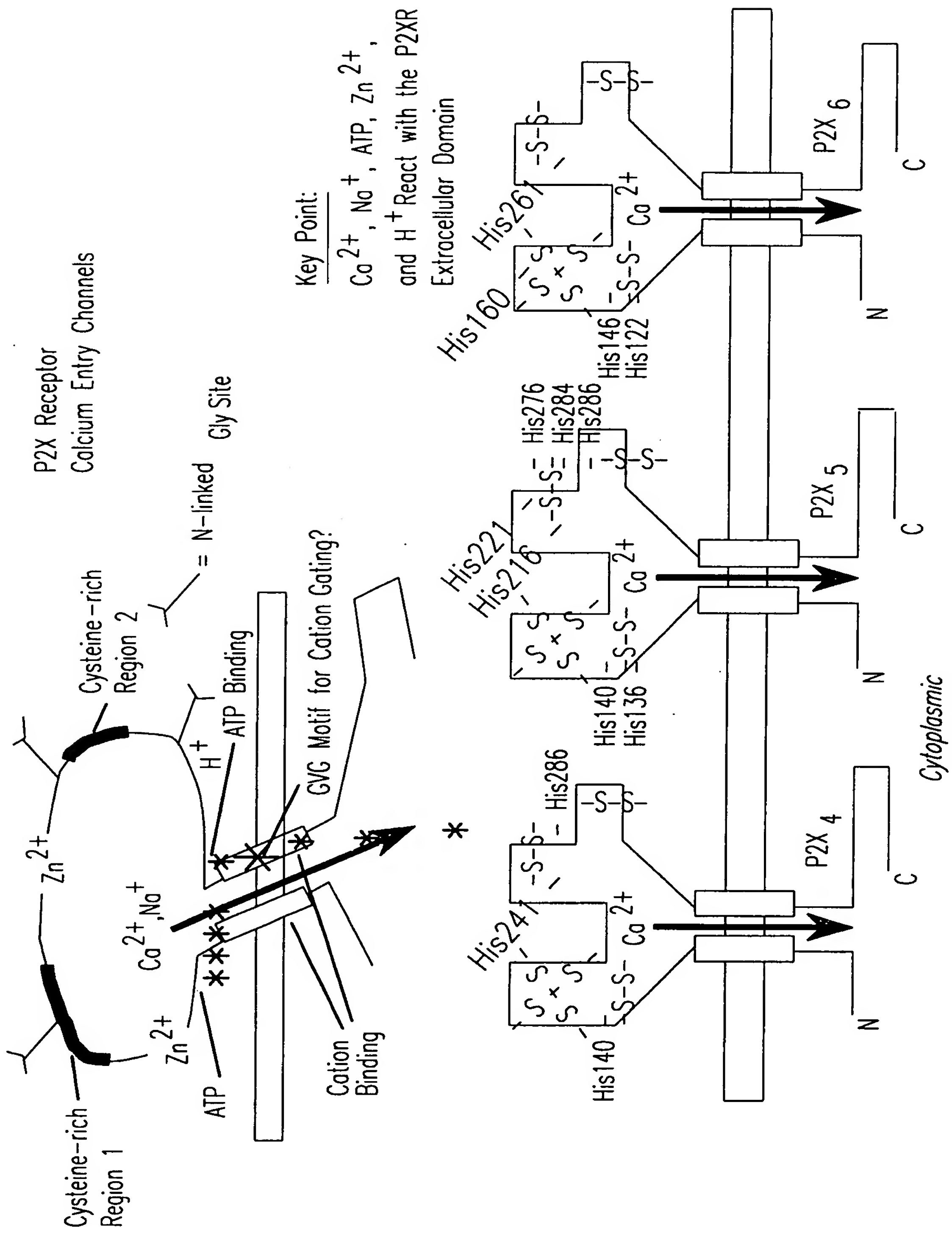

P2X Receptor
Calcium Entry Channels
Cysteine-rich Region 1
Cysteine-rich Region 2
= N-linked Gly Site
Zn^{2+}
Zn^{2+}
Ca^{2+},Na^{+}
H^{+}
ATP Binding
GVG Motif for Cation Gating?
ATP
Cation Binding
Key Point:
Ca^{2+}, Na^{+}, ATP, Zn^{2+},
and H^{+} React with the P2XR
Extracellular Domain
His140
His241
His286
Ca^{2+}
P2X 4
His140
His136
His216
His221
His276
His284
His286
Ca^{2+}
P2X 5
His160
His261
His146
His122
Ca^{2+}
P2X 6
N
C
N
C
N
C
Cytoplasmic

FIG.19A

TRPC Calcium Entry Channels

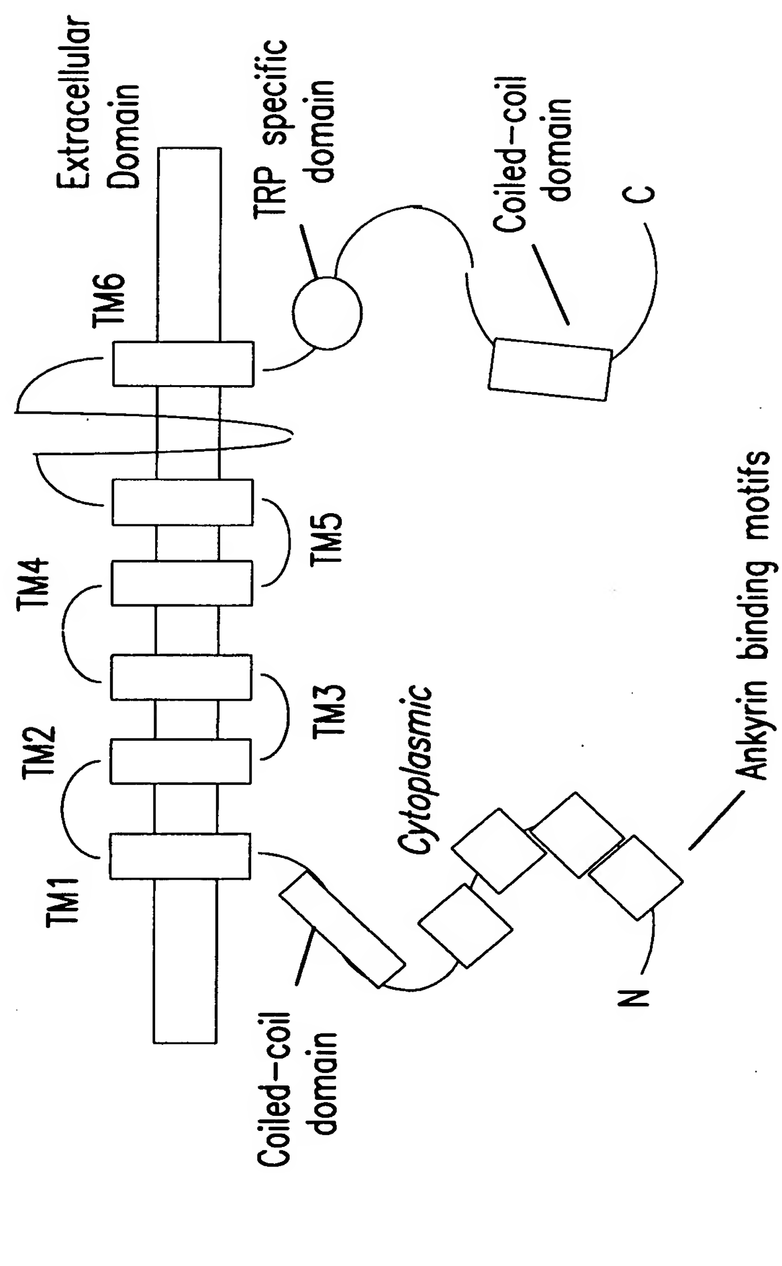


FIG.19B

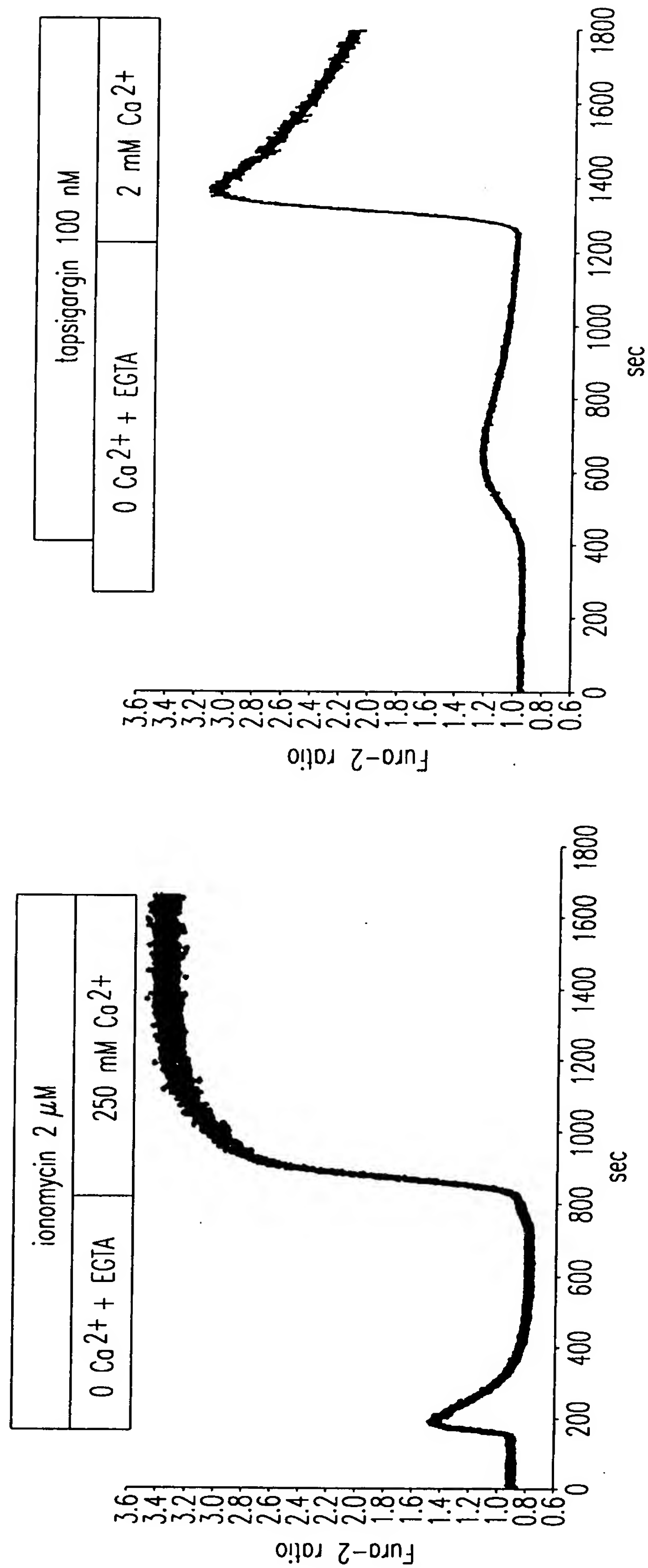
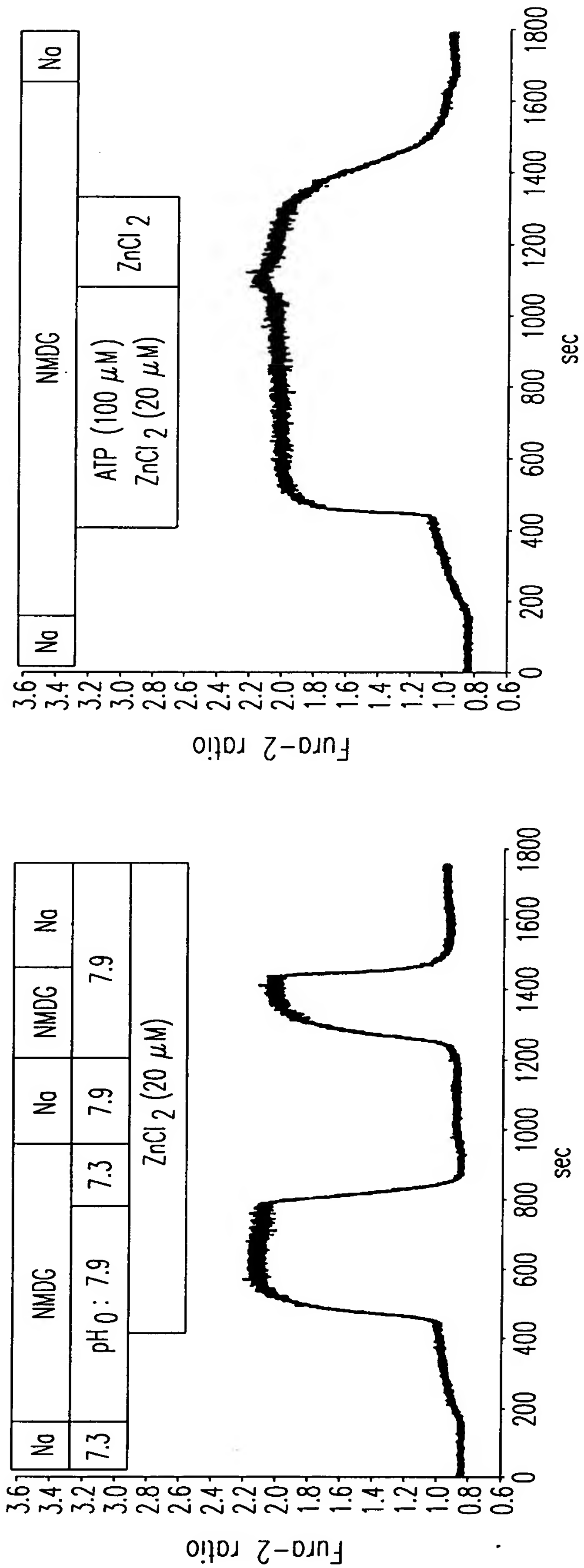

ionomycin 2 μM
0 Ca2+ + EGTA
250 mM Ca2+
Fura-2 ratio
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0
200
400
600
800
1000
1200
1400
1600
1800
sec
topsigargin 100 nM
0 Ca2+ + EGTA
2 mM Ca2+
Fura-2 ratio
sec

FIG.19C-1

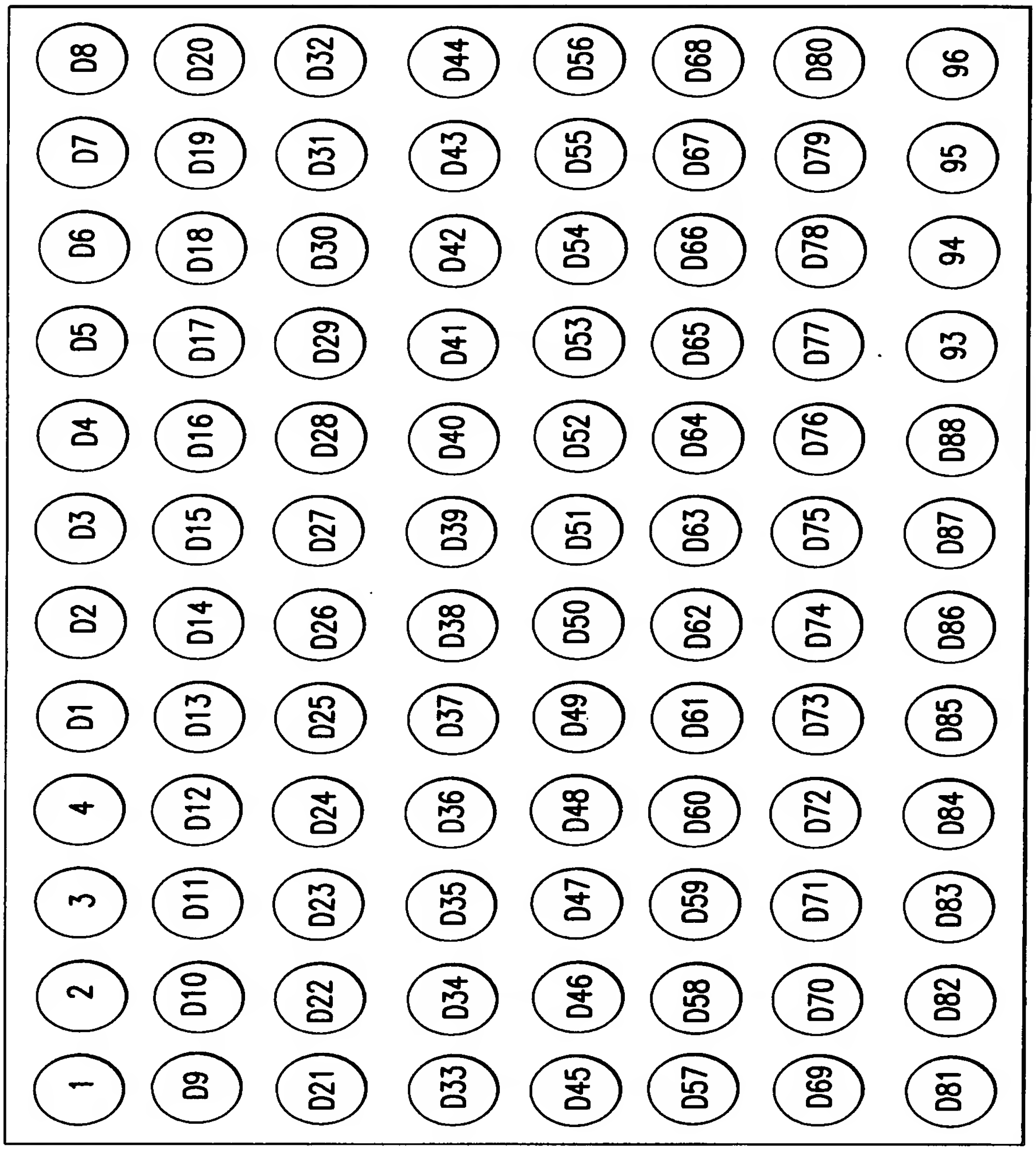


Na
NMDG
ATP (100 μM)
ZnCl 2 (20 μM)
ZnCl 2
Fura-2 ratio
sec
Na
7.3
pH 0 : 7.9
7.3
7.9
NMDG
7.9
ZnCl 2 (20 μM)
Fura-2 ratio
sec

FIG.19C-2

Designation	Mode of Stimulation	Epithelial Polarity
Store-operated Ca^{2+} channels (SOCs) or I_{CRAC}	ER store depletion	Unclear
TRP channels	ER store depletion (partial) Alkaline extracellular pH (partial)	Apical & Basolateral
P2X receptor Ca^{2+} entry channels	Extracellular zinc and ATP	Apical & Basolateral
ECaC or CAT *(Related to TRPs)*	ER store depletion	Apical
Ca^{2+}–permeable non–selective cation channel (NSCC)	Stretch–activated	Apical

FIG.19D



1 2 3 4 D1 D2 D3 D4 D5 D6 D7 D8
D9 D10 D11 D12 D13 D14 D15 D16 D17 D18 D19 D20
D21 D22 D23 D24 D25 D26 D27 D28 D29 D30 D31 D32
D33 D34 D35 D36 D37 D38 D39 D40 D41 D42 D43 D44
D45 D46 D47 D48 D49 D50 D51 D52 D53 D54 D55 D56
D57 D58 D59 D60 D61 D62 D63 D64 D65 D66 D67 D68
D69 D70 D71 D72 D73 D74 D75 D76 D77 D78 D79 D80
D81 D82 D83 D84 D85 D86 D87 D88 93 94 95 96

FIG.20A

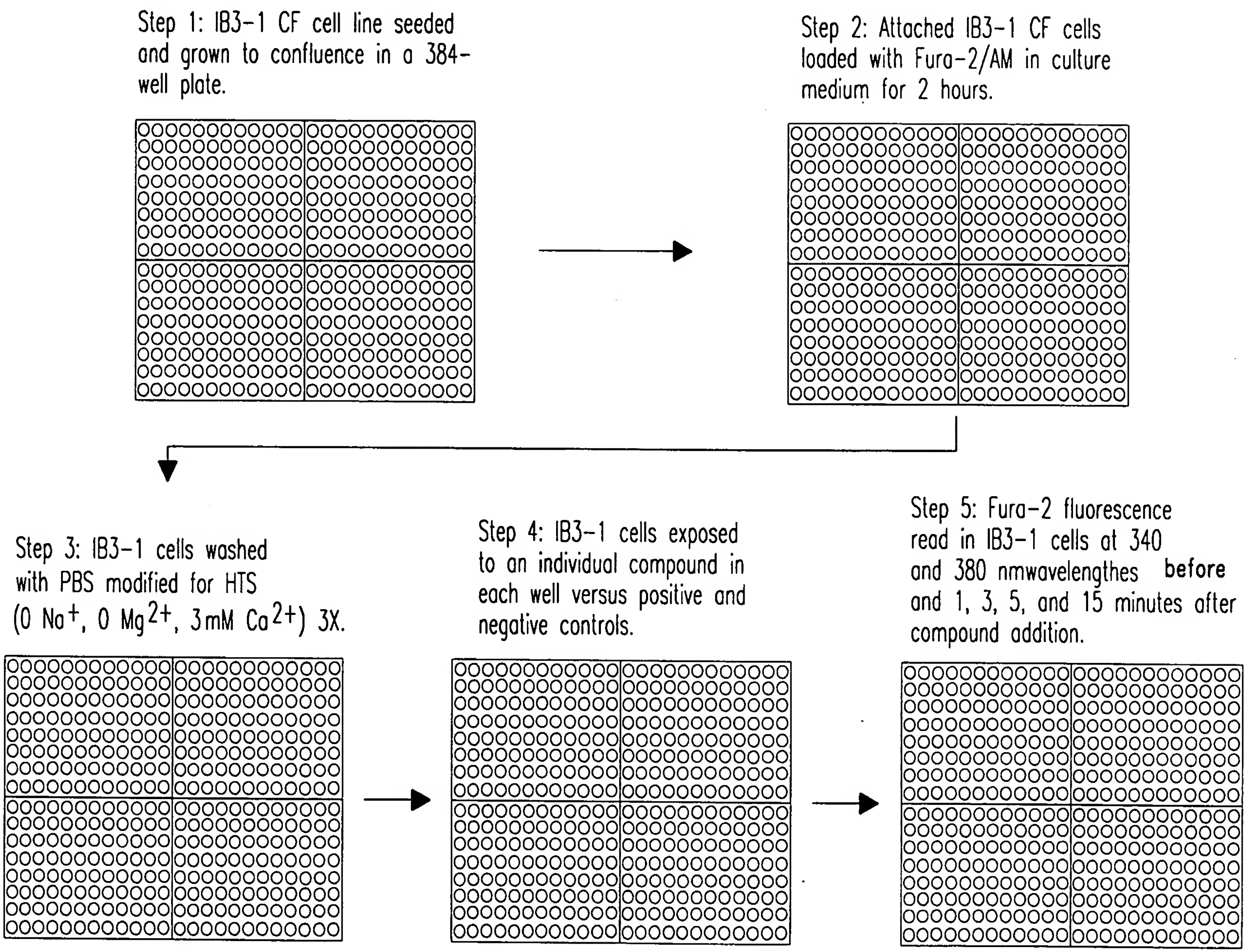

Step 1: IB3-1 CF cell line seeded and grown to confluence in a 384-well plate.
Step 2: Attached IB3-1 CF cells loaded with Fura-2/AM in culture medium for 2 hours.
Step 3: IB3-1 cells washed with PBS modified for HTS (0 Na+, 0 Mg2+, 3mM Ca2+) 3X.
Step 4: IB3-1 cells exposed to an individual compound in each well versus positive and negative controls.
Step 5: Fura-2 fluorescence read in IB3-1 cells at 340 and 380 nmwavelengthes before and 1, 3, 5, and 15 minutes after compound addition.

FIG.20B

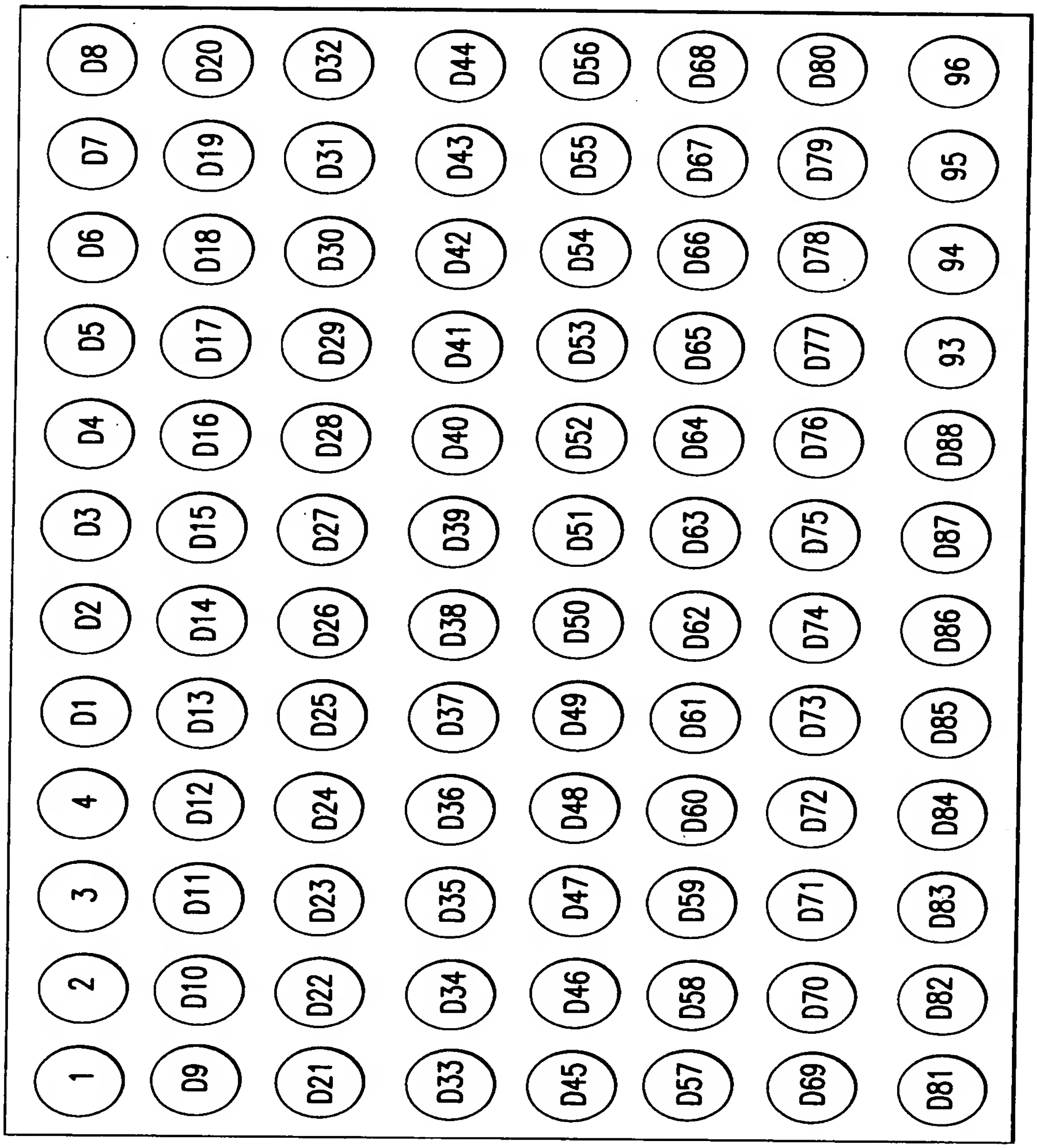

1 2 3 4 D1 D2 D3 D4 D5 D6 D7 D8
D9 D10 D11 D12 D13 D14 D15 D16 D17 D18 D19 D20
D21 D22 D23 D24 D25 D26 D27 D28 D29 D30 D31 D32
D33 D34 D35 D36 D37 D38 D39 D40 D41 D42 D43 D44
D45 D46 D47 D48 D49 D50 D51 D52 D53 D54 D55 D56
D57 D58 D59 D60 D61 D62 D63 D64 D65 D66 D67 D68
D69 D70 D71 D72 D73 D74 D75 D76 D77 D78 D79 D80
D81 D82 D83 D84 D85 D86 D87 D88 93 94 95 96

FIG.20C

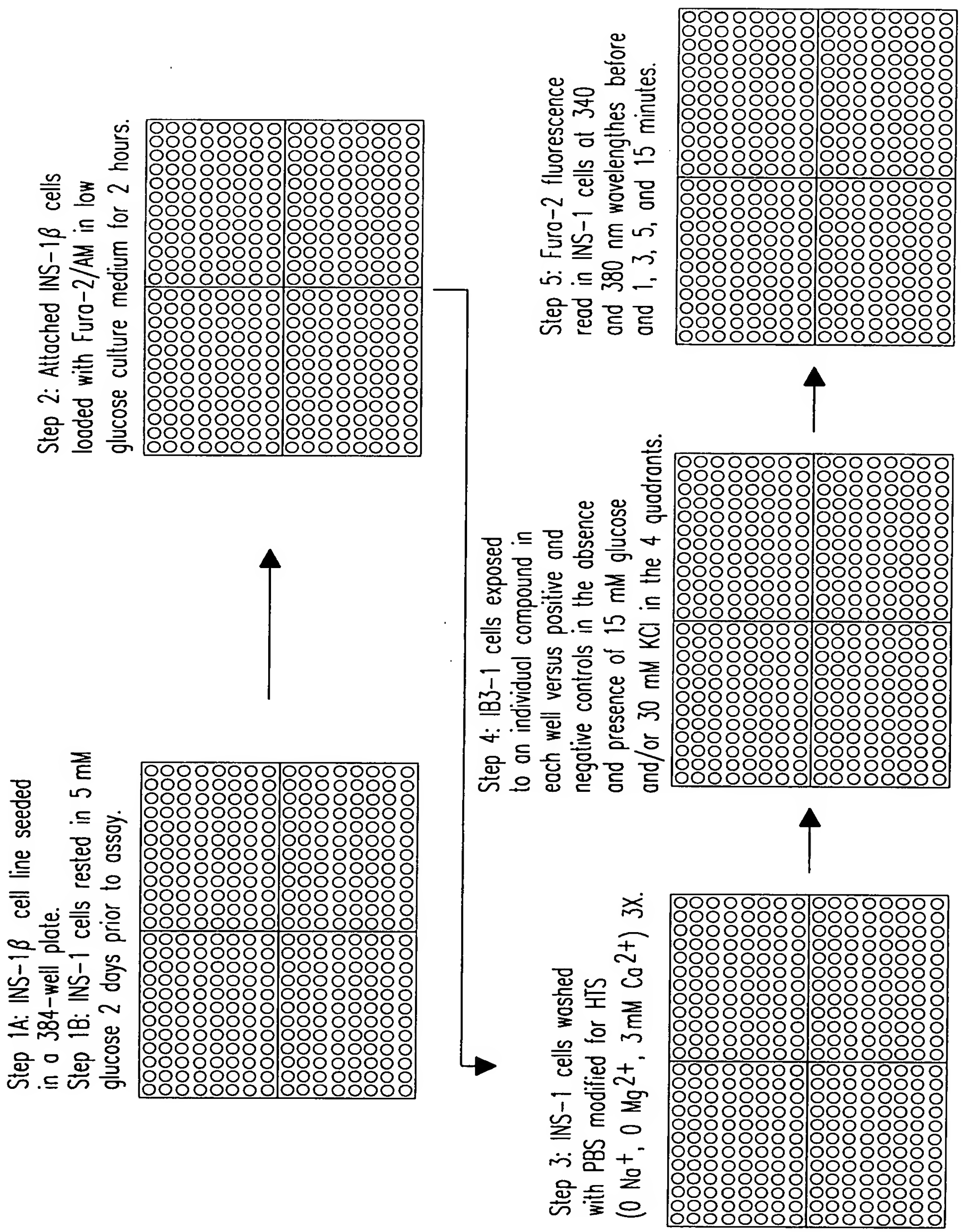

Step 1A: INS-1β cell line seeded in a 384-well plate.
Step 1B: INS-1 cells rested in 5 mM glucose 2 days prior to assay.
Step 2: Attached INS-1β cells loaded with Fura-2/AM in low glucose culture medium for 2 hours.
Step 3: INS-1 cells washed with PBS modified for HTS (0 Na+, 0 Mg2+, 3 mM Ca2+) 3X.
Step 4: IB3-1 cells exposed to an individual compound in each well versus positive and negative controls in the absence and presence of 15 mM glucose and/or 30 mM KCl in the 4 quadrants.
Step 5: Fura-2 fluorescence read in INS-1 cells at 340 and 380 nm wavelengthes before and 1, 3, 5, and 15 minutes.

FIG.20D

Lead Ligand from Human Epithelial Cell HTS for CF

Lead Ligand from Rat β Cell HTS for CF

Secondary Full Definition of the Lead Ligand Effects on Calcium Entry

Battery of Cell Toxicity Screens in Appropriate Cell Models

Secondary Full Definition of the Lead Ligand Effects on Calcium Entry

Rescue of NaCl Transport Dysfunction in CF Epithelia In Vitro

Rescue of Insulin Secretion in the INS-1 β Cell Line

Rescue of NaCl Transport Dysfunction in CF Epithelia In Vivo

Rescue of Insulin Secretion in Islets Isolated and Grown in Primary Culture from Normal and Diabetic Rats

FIG.20E